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Review

Gastrointestinal cancer of the microsatellite mutator phenotype pathway

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Introduction

A novel type of genetic instability, usually designated as microsatellite instability (MSI), is characterized by length alterations within simple repeated sequences. The accumulation of hundreds of thousands of clonal somatic mutations in these neutral sequences is the landmark of cancer of the microsatellite mutator phenotype (MMP).[1] The MMP is characteristic of most hereditary nonpolyposis colorectal cancers (HNPCC). About 10%–15% of unselected gastrointestinal cancers also display this mutator phenotype, including tumors without documented family history (sporadic cancer).[2] Genetic and epigenetic inactivation of DNA mismatch repair (MMR) genes leads to mutations in cancer genes and to cancer development.[3,4] We have proposed that the MMP underlies a distinctive tumorigenic pathway, because gastrointestinal cancers with the MMP exhibit many differences in genotype and phenotype relative to tumors without it, irrespective of their hereditary or sporadic origins.[1,3,5] Thus, MMP colorectal cancers exhibit low frequencies of mutations in the *p53*, K-*ras*, and *APC* genes, prototypical cancer genes in colon tumors of the classical suppressor pathway without MMR deficiency.[1,2,6] The differences in genotype can be explained because MMR deficiency leads to an exacerbated mutator phenotype with a very specific mutation spectrum. The MMP rapidly leads to frameshift mutations in mononucleotide tracts present in genes such as the transforming growth factor (TGF) β receptor type II (*TGFβRII*)[7] and *BAX*.[8] These mutations are absent in cancers that are MMP-negative.[5,7,8] The peculiar genotype of tumors of the MMP also includes specific patterns of gene regulation. For instance, *COX-2* overexpression is less frequent in MMP colorectal cancers. Gastrointestinal cancers of the MMP pathway also display an aberrant epigenetic pattern, such as hypermethylation of some genes, including *hMLH1*, the key mutator MMR gene. The differences in genotype and phenotype between gastrointestinal cancer with and without the MMP are likely to be causally linked to their differences in biological and clinical features. Diagnostic characterization of the MMP status thus has implications in clinical oncology.

"Microsatellite instability" and the concept of ubiquitous somatic mutations

Tumors of the hereditary nonpolyposis colorectal cancer (HNPCC) syndrome and some unselected gastrointestinal tumors belong to the MMP pathway.[9] The MMP accounts for the mutational activation and inactivation of cancer genes (those with positive and negative roles in cell growth or survival), which drive multistep carcinogenesis.[6,10] Tumors of the MMP pathway accumulate hundreds of thousands of somatic mutations in simple repeated sequences or microsatellites.[1] Spontaneous errors of replication due to slippage by strand misalignment[11] are fixed as mutations and accumulate because of defects in replication fidelity of these unstable sequences, if the DNA mismatch repair (MMR) machinery fails (Fig. 1).[12] The discovery of the MMP, by the detection of these ubiquitous somatic mutations, provided conclusive evidence for the hypothesis of cancer as a mutator phenotype.[13]

Microsatellite instability (MSI) is frequently used to describe the genomic instability underlying the patho-

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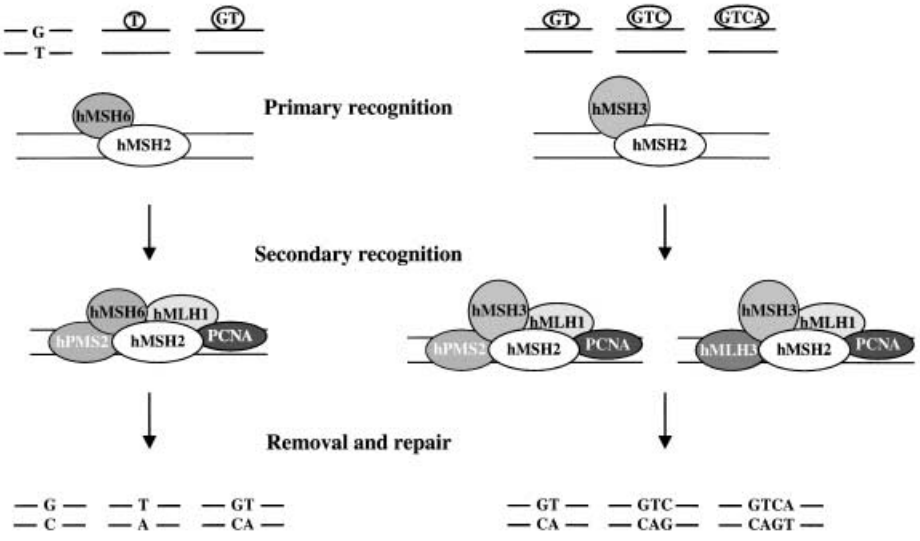

Fig. 1. A model of DNA mismatch repair

genesis of HNPCC and other MMP tumors. However, MSI may not be an accurate definition, because these sequences are intrinsically unstable. Detection in tumors of sporadic alterations in microsatellite sequences is not necessarily diagnostic of genomic instability, because they could be spontaneous errors of replication of these unstable sequences in the absence of any defects in the cellular replication machinery. These mutations are detected in the tumors because their clonal expansion unveils them, and they are otherwise invisible in the polyclonal normal tissue. Microsatellite mutations are thus useful as markers of clonality[14] or of mitotic activity,[15] but they may be completely unrelated to the MMP.

The distinction between "true" instability and clonality is diagnostic of two distinctive molecular pathways for gastrointestinal cancer. Microsatellite clonality in the absence of instability is diagnostic of the classical tumor suppressor pathway for aneuploid cancer.[6] "True" MSI is, on the other hand, diagnostic of the MMP pathway for (pseudo)diploid gastrointestinal cancer.[12,16]

Tumors in the suppressor pathway may derail the homeostatic control of gene expression that is, presumably, required for tumor development, by altering the chromosomal balance. This not only unmasks recessive tumor suppressor genes but also increases the amounts of other cancer gene products with positive roles in cell growth or survival.[17] In contrast, tumors with the MMP may achieve the same alteration of overall patterns of gene expression by the sheer numbers of frameshift and other (point) mutations. These mutations not only occur in coding regions of genes but also in regulatory gene regions.[18] One example has been already reported, showing that intronic mutations in the splicing donor site of one of the exons of the *ATM* gene alter the splicing of the gene, thus influencing its expression.[19] A similar situation is found in another gene involved in genome integrity, the *MRE11* (Giuseppe Giannini, personal communication).

The diagnostic detection of the MMP in gastrointestinal tumors is of value in the clinical arena, because it may enable the detection of hereditary cases. In addition, it has prognostic value, because tumors in the mutator pathway appear less aggressive than those in the suppressor pathway.[5]

Criteria for classification of gastrointestinal cancer with MMP

The criteria of MSI for colorectal cancers proposed by the National Cancer Institute (NCI) workshop[3,20] are summarized in Table 1. Analysis of a panel of 381 unselected colorectal tumors yielded 46 MMP+ (high-frequency MSI; MSI-H) (12%), 36 MMP+/− (low-frequency MSI; MSI-L) (9%), and 299 MMP− (microsatellite stable; MSS) (79%) tumors. The results

Table 1. Criteria of MSI for colorectal cancers proposed by the National Cancer Institute workshop[3,20]

	No. of markers exhibiting instability length changes	
	5 Loci analyzed	>5 Loci analyzed
MSI-H	≧2	≧30%–40%
MSI-L	1	<30%–40%
MSS or MSI-L	0	0

Reference panel: BAT25, BAT26, D5S346, D2S123, D17S250
MSI, Microsatellite instability; H, high frequency; L, low frequency; MSS, microsatellite stable

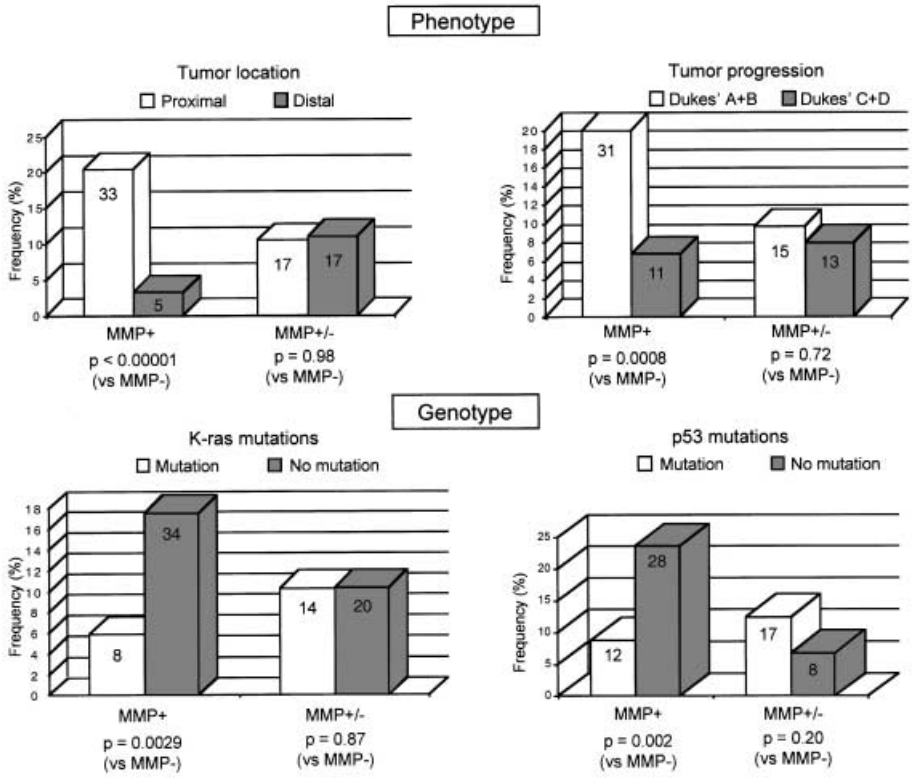

Fig. 2. Comparative features of colorectal tumors according to their microsatellite mutator phenotype (*MMP*) status. The percent values shown in the MMP+ vs MMP+/− series reflect the proportions of represented cases versus the rest of the cases. The probability values were calculated by the χ^2 with Yates correction, or by the Fisher exact test

are summarized in Fig. 2. Analysis of the frequency of mutations in target genes for MMP revealed that they were absent in MMP+/− tumors (Fig. 3). Similar results were also obtained in gastric tumors.[5,21] The conclusions from these findings are that MMP tumors differ from the other gastrointestinal tumors in most clinical, biological, and molecular parameters. Therefore, microsatellite alterations in MMP tumors represent true genomic instability underlying a mutator pathway for cancer. Tumors with the MMP are distributed unequally along the gastrointestinal tract (Fig. 4), although the reasons for this asymmetry are not well understood.

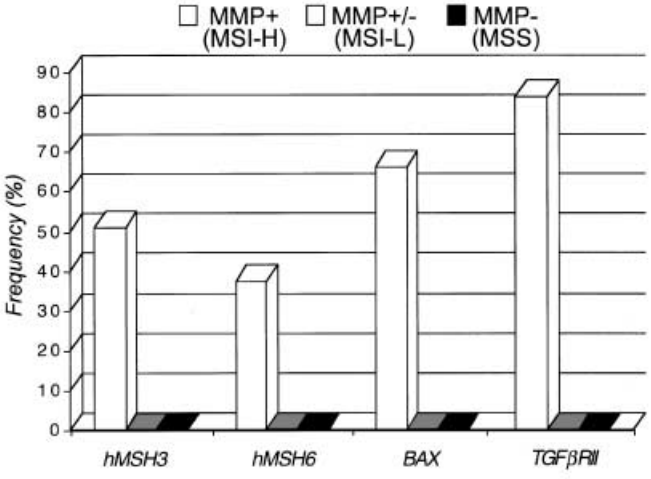

Fig. 3. MMP+/− tumors do not accumulate mutations in the MMP target cancer genes. *MSI-H*, Microsatellite instability-high frequency; *MSI-L*, MSI-low frequency; *MSS*, microsatellite stable

MMP+/− or MSI-L and hMSH6 mutations

MMP+/− (MSI-L) tumors are, on the other hand, indistinguishable from those without microsatellite alterations in every parameter we analyzed. Therefore, these isolated microsatellite alterations, although useful markers of clonality or mitotic activity, do not appear to represent indicators of genomic instability. The mutations observed in MMP+/− tumors may represent a background level of genetic instability present in all gastrointestinal tumors and their precursor normal cells. If a sufficient number of markers are analyzed (we estimate the number to be from 150 to 200), all tumors would exhibit MSI-L according to the criteria for classification by the NCI workshop (i.e., mutations in one dinucleotide locus). Indeed, it was reported that this was the case for Barrett's-associated esophageal adenocarcinoma.[22]

Whether MSI-L tumors may be composed of two groups, a group indistinguishable from MSS (microsatellite stable) tumors, and another distinct group that may have a higher number of mutations

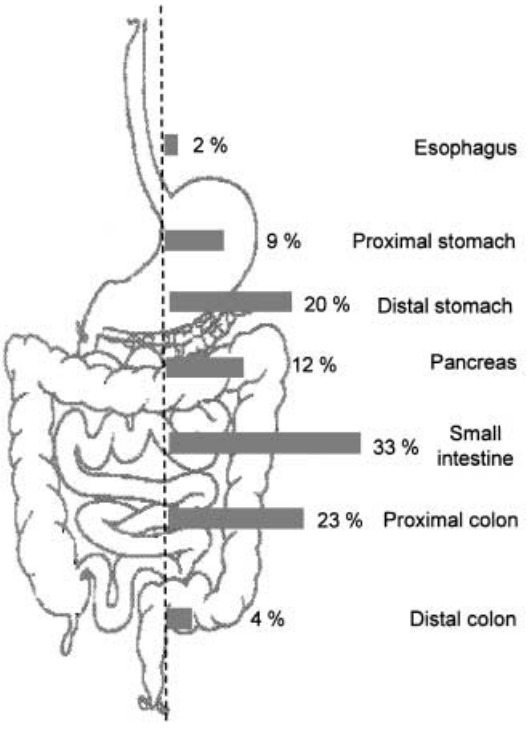

Fig. 4. Incidence of the MMP in cancers of the gastrointestinal tract

due to some low or transient instability, remains to be demonstrated. The nature of MMP+/− has not yet been substantiated by characterization of underlying DNA MMR or other defects.[3] The main problem resides in the difficulty in establishing a criterion for the distinction of these putative "true" MSI-L tumors from the rest of the MSS (or "false" MSI-L tumors) based on the number of dinucleotide microsatellite loci alterations.

Whitehall et al.[23] suggest that silencing of *MGMT* predisposes to mutation by overwhelming the DNA MMR system, and this occurs with greatest frequency in MMP+/− colorectal cancers. A frequent loss of imprinting of the insulin-like growth factor II (*IGFII*) gene has been reported in colorectal cancer tissues, as well as in the matched normal colonic mucosa of patients with MMP+ or MMP+/− cancer.[24] If these reports are corroborated, there will be an urgent need to set the diagnostic features to distinguish the "true" MSI-L from the "false" MSI-L (or MSS). One obvious possibility would use the specificity of mononucleotide repeats instability in MSI-H- or MMP-positive tumors, and it is to be hoped, the discovery of any dinucleotide loci that would be exclusively altered in the MSI-L tumors, but not in MSS tumors. Otherwise, the diagnostic classification based on the number of altered dinucleotide loci will irremediably lead to artificial cutoff points of difficult validation.

In a population-based study of early-onset colorectal cancer (<50 years), Verma et al.[25] have identified a subgroup of tumors with MSI for mono-, but not dinucleotide repeat markers (m-MSI+ group). The m-MSI+ group cancers were mainly left-sided (6/7). They have identified a germline *hMSH6* mutation in an isolated case of early-onset colorectal cancer (43 years). Plaschke et al.[26] have identified a germline *hMSH6* mutation accompanied by a somatic mutation in an m-MSI+ tumor from an HNPCC-like patient. Kolodner et al.[27] have found germline *hMSH6* mutations in 6 of 91 population-based familial non-HNPCC individuals, suggesting that germline *hMSH6* mutations predispose individuals to primarily late-onset, familial colorectal carcinomas that do not fulfill classic criteria for HNPCC. Wu et al.[28] have detected four presumably causative *hMSH6* mutations in 4 of 18 patients who had suspected HNPCC and MSI-L tumors. In contrast, Parc et al.[29] have found only one somatic mutation in 41 sporadic tumors with MSI-L, suggesting that *hMSH6* mutations do not play a major role in the development of sporadic colorectal cancer with MSI-L. We have obtained results similar to those of Parc et al.,[29] supporting the role of *hMSH6* as primary mutator in some hereditary and sporadic cancers, but without correlation between *hMSH6* mutations and MSI-L.[30]

Gastrointestinal cancer pathways

Two apparently mutually exclusive genomic instabilities define two distinct pathways for gastrointestinal cancer.[12,16] Chromosomal instability is associated with the suppressor pathway for aneuploid cancer, and the MMP underlies the mutator pathway for (pseudo)diploid cancer. The main difference distinguishing the suppressor from the mutator pathway is that a tumor suppressor gene mutation leads to growth or territorial expansion advantage, while a mutator mutation does not (Fig. 5). The tumor suppressor pathway usually involves mutations in the tumor suppressor genes *APC* and *p53* and the oncogene K-*ras*.[8] The mutator phenotype pathway[16] unfolds after a mutation occurs in a mutator gene (i.e., DNA MMR family). The MMP represents a distinctive molecular pathway for gastrointestinal cancer, because the cancer genes mutated in MMP+ tumors are generally different from those mutated in tumors in the suppressor pathway.[3,12,31,32] This hypothesis originated from the observation that colon tumors with the MMP displayed paradoxically low mutation frequencies for the two prototypical examples of cancer genes, the *c-K-ras* oncogene and the *p53* tumor suppressor gene.[1] Therefore, while the "distal" molecular genetic cause (the mutator mutations) of cancer with MMP[1] was soon

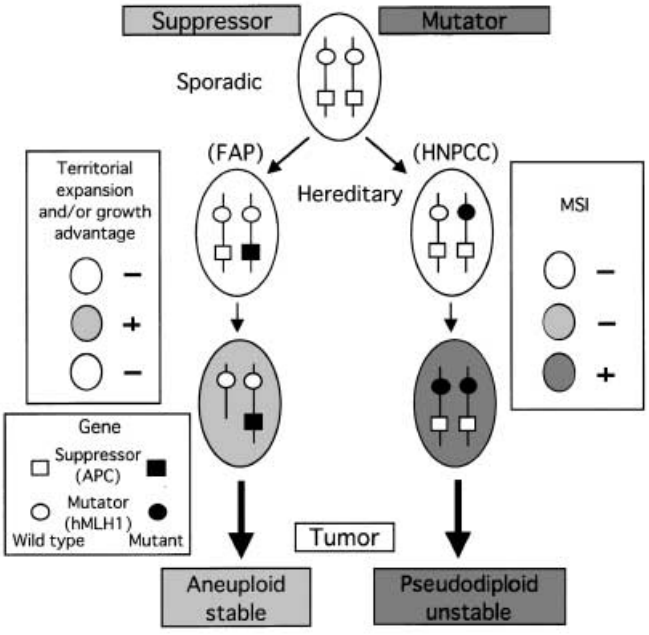

Fig. 5. Genetic pathways for gastrointestinal cancer. *FAP*, Familial adenomotons polyposis; *HNPCC*, hereditary nonpolyposis colorectal cancer

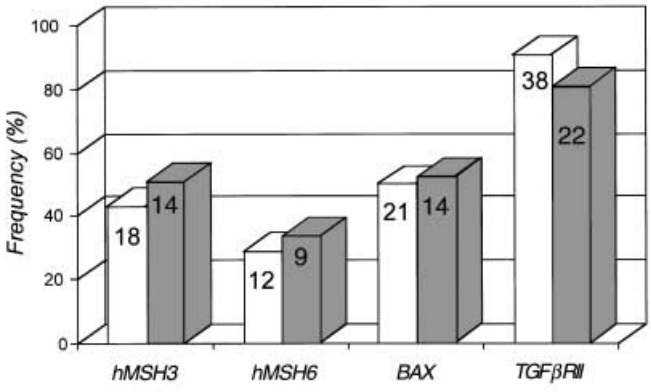

Fig. 6. Hereditary (HNPCC; *gray bars*) and sporadic tumors (*white bars*) with the MMP are identical in genotype

confirmed,[9] the "proximal" cause of the development of cancer with MMP was found later.

Gastric tumors of the MMP are associated with intestinal type, distal location, and better survival, and these tumors exhibit a significantly lower incidence of *p53* gene mutations than the rest of the tumors, suggesting that gastric tumors of the MMP also represent a distinctive oncogenic pathway.[5,21] Pancreatic cancers with the MMP also appear to follow a distinctive oncogenic pathway, because they exhibit peculiar clinical, pathological, and molecular characteristics. Pancreatic cancer with the MMP is associated with poor differentiation, longer overall survival time, and the presence of wild-type K-*ras* and *p53* genes.[33] In contrast, MMP due to defective DNA MMR appears to play little, if any, part in hepatocarcinogenesis.[34] An obvious common denominator between tumors displaying the MMP is the high cell turnover and long mitotic history of the precursor intestinal stem cells, which is, on the other hand, conspicuously absent in tumors from organs that have not been found to exhibit the MMP, such as liver cancer.

Mechanisms for inactivation of DNA MMR genes

Tumors of the mutator and suppressor pathways follow Knudson's "two-hit" model. In hereditary cancers, one mutation is present in the germline and the other is somatic, while both mutations are somatic in sporadic cases. In both pathways for gastrointestinal cancer, inactivation of suppressor or mutator alleles may also be achieved not by mutations but by epimutations. Thus, inactivation of the *hMLH1* mutator gene is often accomplished by an epigenetic alteration, associated with the hypermethylation of its promoter.[4,5,35] The *APC* gene may also be inactivated by DNA hypermethylation, although the relative proportions by which these key genes appear to be inactivated by hypermethylation is clearly asymmetric. There is more involvement of epigenetic inactivation of the *hMLH1* gene in the mutator pathway than of the *APC* gene in the suppressor pathway. Involvement of epigenetic or genetic inactivation of the *hMLH1* gene has also been shown in pancreatic cancer with the MMP.[33]

While the clear differences in phenotype and genotype of tumors with and without the MMP provided the rationale for distinguishing these two pathways for carcinogenesis,[1] hereditary or sporadic tumors of the MMP were essentially indistinguishable in all molecular genetic parameters we analyzed (Fig. 6). If these tumors have the same genomic phenotype (hundreds of thousands of somatic clonal microsatellite mutations), it would be surprising if they were to have significant differences in their cellular genotype or their tumor phenotype.

Late onset and high incidence in females of colon cancer of the MMP with hypermethylated hMLH1 gene

We found that, in the MMP pathway, colorectal tumors with methylated *hMLH1* were distinct from the rest in terms of delayed onset and association with the female sex.[4] MMP+ tumors with the methylated *hMLH1* gene promoter occurred in patients about 18 years older than those without. These MMP+ tumors with *hMLH1* methylation were also about twice as frequent in females than in males. This finding is consistent with previous

observations reporting a higher incidence of MMP+ tumors in older females,[36,37] and it establishes a link between *hMLH1* methylation and the female sex. This link can be explained by sex-specific genetic factors (for instance, a chromosome X-linked gene) or by nongenetic factors. In this context, Slattery et al.[38] evaluated sex-specific differences in the prevalence of MSI in colon tumors, and determined whether reproductive history and hormonal exposure were associated with MSI. They found that the excess of MSI+ tumors in females correlated with an excess of MSI+ tumors at an older age. They suggest that estrogen exposure in females protects against MSI, whereas the lack of estrogen in older females increases the risk of instability. However, this hypothetical explanation needs confirmatory experimental evidence of a mechanism linking estrogen with MSI.

There is no compelling reason why stable epigenetic alterations need to be produced by epigenetic, rather than by genetic events. The late onset of MMP colon cancer in females could be explained by the additional genetic and epigenetic steps (not immediately affecting cell growth or survival) that appear to be involved in this particular pathway for tumorigenesis.[4]

Target cancer genes for MMP

The cancer genes mutated in cancer with MMP are beginning to be characterized. *TGFβRII* and the proapoptotic gene *BAX* are frequently inactivated by slippage-induced frameshift mutations in mononucleotide tracts present in their gene coding regions.[6,7] These findings have provided proof for the causal link between MMP and mutations in cancer genes, and they were also persuasive examples of the differences between the mutator and suppressor pathways for cancer. In contrast with the high incidence of *TGFβRII* and *BAX* frameshift mutations in MSI-H tumors, these mutations are absent in tumors in the suppressor pathway. These genes have also been found to be mutated in tumors of the suppressor pathway, but with a lower frequency and not by slippage-linked frameshifts.[39,40]

The *BAX* gene has, in its amino terminus, a run of 8 Gs, which is a target for MMP, generating frameshift mutations inactivating the gene product (Fig. 7). These frameshift mutations are frequent in MMP gastrointestinal cancers.[5,8,20,39,41,42] The high incidence of *BAX* frameshift mutations in MMP+ tumors and their absence in MMP-tumors suggest that these mutations are under a selective pressure during tumor progression in the mutator pathway. This hypothesis was supported by the absence or very low frequency of frameshift mutations in identical repeated sequences present in other genes.[39,41–43]

The identification of *BAX* mutations also helped to explain the paradoxical low *p53* mutation frequency in the MMP pathway for cancer. p53 is a transcription activator, and *BAX* is one of its targets. Bax mediates the apoptotic signaling by p53. However, in the presence of frameshift *BAX* mutations, its transcriptional activation by p53 in response to DNA damage would be futile.[5,8,39] Once MMP unfolds, the mutational events leading to cancer are stochastic, but predictable, because mutations in the *BAX* slippage hotspot usually occur sooner than those in the *p53* gene, which lacks such repeats.

Several *BAX* missense mutations, with a "hotspot" of transitions at codon 169, have been reported in gastrointestinal tumors with MMP.[39] Gil et al.[44] replaced the threonine at this position by an alanine or by a methionine, and they have shown that both missense

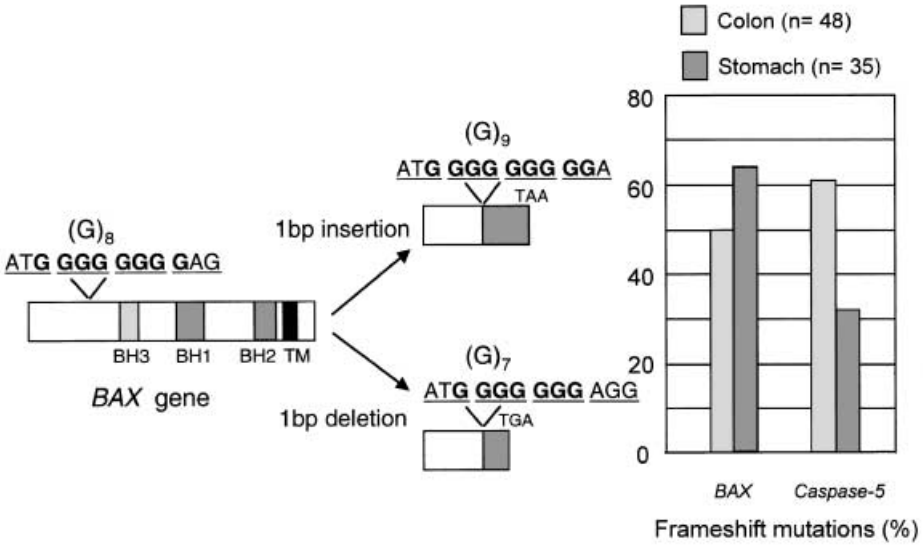

Fig. 7. Frameshift mutations are frequent in the proapoptotic gene *BAX* in MMP tumors

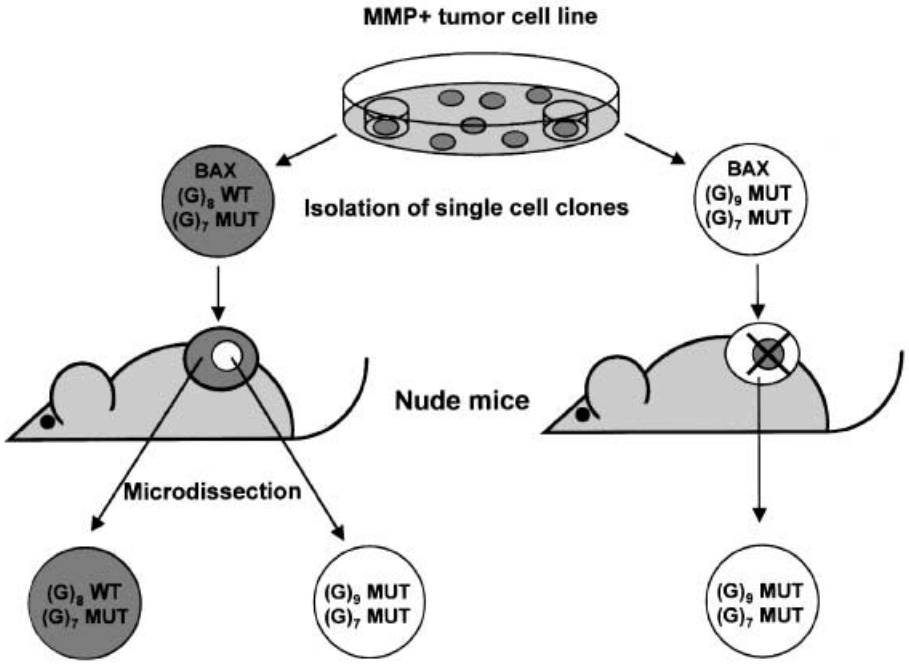

Fig. 8. *BAX* mutational inactivation is under selective pressure during tumorigenesis. Single cell clones were isolated from some tumor cell lines of the mutator phenotype, heterozygous for the *BAX* frameshift mutation. The cells contain both the normal, 8G allele, and the mutant, 7G allele. One to 2 months after inoculation, the tumors that developed were composed mostly of heterozygous cells that were visualized by staining with an anti-Bax antibody. But, very often, there were clones in the tumor that had lost the wild-type allele, and they were homozygous, with only mutant alleles, and were not stained by the Bax antibody. In contrast, parallel experiments inoculating single cell clones with homozygous *BAX* mutations (G9 and G7) into the animals did not produce in vivo clones heterozygous for *BAX* frameshift mutations. These findings imply that there is a strong selection for *BAX* mutational inactivation during in vivo tumorigenesis

mutations at codon 169 of *BAX* are functional, because they inhibited its apoptotic activity. This is the first report of the functional significance of missense mutations in *BAX*, or any other proapoptotic member of the Bcl-2 family, in primary human tumors.

It has also been shown that inactivation of the wild-type *BAX* allele by de-novo frameshift mutations confers strong advantage during tumor clonal evolution (Fig. 8).[43] These results support the interpretation that *BAX* inactivation contributes to tumor progression by providing survival advantage. In this context, survival analyses show that *BAX* mutations are indicators of poor prognosis for both colon and gastric cancer of the MMP.[43] It has recently been shown that tumor cells with MMP easily develop resistance to nonsteroidal anti-inflammatory drugs through an inherent instability in the mononucleotide tract in *BAX*.[45]

The mutator that mutates other mutators

Due to the still limited knowledge of the human genome and the strong mutator phenotype of MMP tumors, it is likely that there are many genes mutated in cancer of the MMP.[46] The targets for MMP are not only cancer genes, such as *TGFβRII* or *BAX*, but other mutator genes as well. The model of the "mutator that mutates another mutator"[47] was proposed because of the detection of frequent frameshift mutations in mononucleotide tracts present in the coding region of *hMSH3* and *hMSH6* DNA MMR genes.[39,48] These secondary mutators are probably inactivated by the mutagenic effect of primary mutators.[41,42] The secondary mutator mutations may increase the depth or width of the tumor cell genomic instability, accelerating tumor progression. Functional evidence supporting this model has been found in single-cell clones of the colon cancer cell line SW48, harboring one or two mutators. *hMSH6*(−/−) cells, which have inactivated *hMSH6* in addition to unexpressed *hMLH1*, display a different spectrum of mutations, and a mutation rate about 2.5 times higher than that in *hMSH6*(+/+) cells. This implies that *hMLH1* may not be required for all MutL activity in human cells and that an alternative pathway for the MutL function has about equal capacity. Therefore, the current model for the mechanism of MMR may need to be reevaluated.[48a]

β2-Microglobulin gene mutations and unfavorable prognosis in MMP+ cancers

Gastrointestinal cancers of the mutator pathway are less aggressive than the rest.[5,49–51] The good prognosis of patients with gastric and colorectal cancers of the MMP remains unexplained. In these tumors, MMR deficiency generates many aberrant proteins (i.e., truncated by frameshift mutations),[5,8] providing a source of abnormal peptides that can be presented to cytotoxic T lymphocytes. Thus, tumors of the MMP may be highly immunogenic. In this regard, it is of interest that prominent lymphoid infiltration has been shown in MMP+ cancers.[49]

Inactivating mutations in the *HLA* antigen and β2-microglobulin (*β2M*) genes, which are required for peptide presentation, is one mechanism by which cancer cells may escape immune recognition by cytotoxic T cells.[52] Frequent *β2M* mutations have been found in tumors of the MMP, suggesting that these tumors are under selective pressure for obliterating antigen presentation.[53,54] It has recently been reported that *β2M* mutation is a frequent event, not only in gastric cancers but also in sporadic and hereditary colorectal cancers of the mutator phenotype.[55] Moreover, *β2M* mutation was associated with unfavorable prognosis in patients with cancer of the MMP pathway.[5,55] These and previous results[53,54] show that genes indirectly involved in tumorigenesis (i.e., by contributing to the escape from the immune response) can be mutational targets for the MMP, despite not immediately affecting cell growth or survival. As for *BAX* mutation,[43] *β2M* mutation defines useful differences in genotype and phenotype among cancers of the mutator phenotype. These findings are also pertinent to the previous observations of a favorable impact of the MMP in gastrointestinal cancer outcome,[5,49,50] because they establish a link between the MMP and a strong immune response.

Accumulative haploinsufficiency model

The MMP pathway for gastrointestinal cancer presents several paradoxical features.

First, despite accumulating hundreds of thousands of clonal somatic mutations in simple repeated sequences, these tumors exhibit a low mutation incidence in *APC*, K-*ras*, and *p53* prototypical cancer genes for carcinogenesis.[1,6] This first paradox may be explained by the existence within some genes of simple repeats that are preferred targets for MMP. Thus, in the presence of the mutator phenotype, mutations in these genes (e.g., *BAX*) occur *sooner* than in other genes of the same oncogenic (i.e., apoptotic) signaling pathways that do not have these repeats (e.g., *p53*).[8]

Related to this feature, MMP tumors usually display mutation frequencies in cancer genes lower than the frequencies displayed by tumors of the suppressor path-

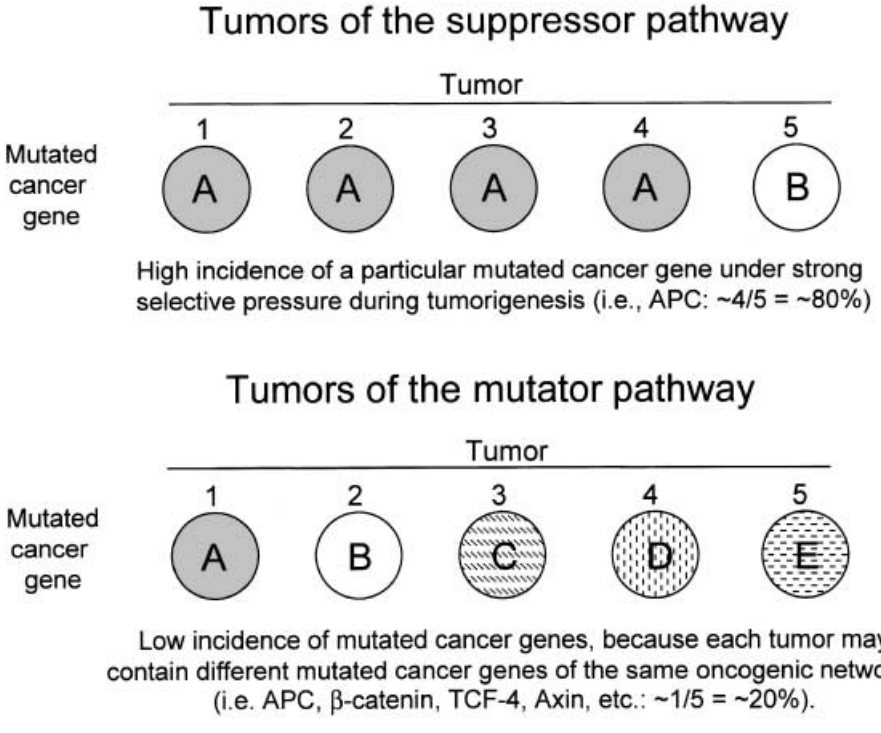

Fig. 9. Mutated cancer gene spectrum in tumors

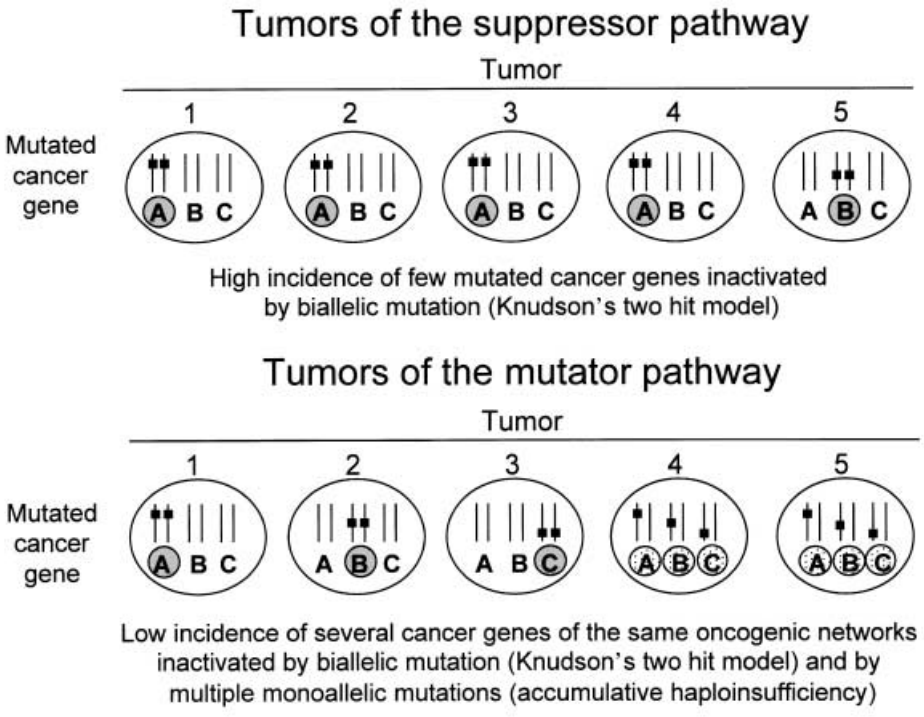

Fig. 10. Accumulative haploinsufficiency model

way. The only known exception is the *TGFβRII* gene, with mutation incidences that, in colon cancer of the MMP, reach nearly 90%. This may be due to the relatively long intragenic $(A)_{10}$ repeat, as well as to a relatively strong selective pressure for inactivation of the transforming growth factor (TGF) network. The rest of the genes found mutated in MMP tumors exhibit lower mutation frequencies, often being less than 25%. One explanation for this feature is depicted in Fig. 9. Due to the mutator phenotype, MMP tumor cells may accumulate mutations in several individual members of the same oncogenic networks. If the APC/β-catenin signaling network needs to be inactivated in colon cancer, in tumors that are MMP-negative, this is usually achieved by mutations in *APC*. But in MMP-positive tumors, this is achieved sometimes by mutation in *APC*, some other times by mutations in β-*catenin*, and some other times by mutations in other members of the same signaling network, such as *Axin* or *TCF-4*. Thus, in cancer of the mutator pathway, a high incidence of mutations in cancer genes is no longer a required criterion for their functionality. As a corollary, the detection of few mutations in any particular cancer gene may not be taken as evidence for their lack of relevance.

The manifestation of the tumor phenotype by cancers without the MMP is usually associated with the biallelic mutational inactivation of a few cancer genes, such as the *APC* and *p53* tumor suppressors.[6] Indeed, due to their rarity, the finding of biallelic mutations in genes was perhaps the strongest criterion for estimating their oncogenic significance. The last paradox presented by tumors of the MMP is that while the ubiquitous mutations in nonfunctional poly (A)n sequences (such as the poly A tails of the *Alu* repeats), are biallelic,[1] these tumors also accumulate many monoallelic (i.e., heterozygous) mutations in functional sequences, such as the coding regions of mutator (*hMSH3* and *hMSH6*),[48] suppressor (*TGFβRII*),[7] and apoptotic (*BAX*)[8] genes.

We have proposed a model to explain this last paradox (Fig. 10).[46] Due to the exacerbated mutator phenotype of these tumors, their ability to escape apoptosis may be facilitated by the accumulation of heterozygous mutations in multiple genes whose products play partially redundant and partially synergistic roles at different points of the apoptotic signaling network. This accumulation of heterozygous mutations presumably reduces the homeostatic threshold amount of the corresponding proapoptotic gene products. This accumulative haploinsufficiency model is not restricted to apoptotic pathways, but also applies to other networks involved in the homeostatic control of genome integrity and cell proliferation. For instance, the APC/β-catenin network. This model is supported by a recent report describing frequent frameshift mutations in the DNA repair *hRAD50* gene in gastric and colon cancers of the MMP.[56]

Conclusion

The presence of simple repeated sequences in subsets of cancer genes, in concert with defective machinery to

correct their spontaneous slippage-induced mutations, appears to be the ultimate reason for the existence of the suppressor and mutator pathways for cancer. Once the mutator phenotype is manifested, the mutations in cancer genes with slippage targets occur *sooner* than those in cancer genes without targets, which are involved in tumors of the suppressor pathway with no preference for mutational hotspots for MSI.

The accumulative haploinsufficiency model for cancer of the mutator pathway is not restricted to genes affecting cell growth or survival, but may also extend to genes involved in genome integrity, including the MMR genes themselves. Accumulation of monoallelic mutations can lead to MMR function haploinsufficiency originating a weak mutator phenotype. This would increase with additional MMR mutations until reaching a "maximum" mutator phenotype, after which no further "delayed" selection would occur. This scenario is particularly relevant to tumors involving the incomplete or gradual inactivation of the initial mutator, such as splicing mutations, or to gradual epigenetic MMR silencing.

This model leads to another curious situation, because when a "maximum" mutator phenotype is reached, the probability of occurrence of nonfunctional and inconsequential mutations is also increased. Therefore, it is difficult to determine in a primary tumor which of the DNA MMR or other DNA repair gene mutations are functional and which are neutral. This argument obviously also applies to cancer genes. The difficulty is magnified because the high mutation rates in MMP tumors depreciate the presence of a gene mutation as a criterion for its functionality.[57] However, as shown in Figs. 9 and 10, the criterion for a high mutation frequency of a gene may no longer apply to MMP tumors, because these tumor cells may also accumulate mutations in several genes of the same oncogenic networks.

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