Frameshift Mutations at Mononucleotide Repeats in caspase-5 and Other Target Genes in Endometrial and Gastrointestinal Cancer of the Microsatellite Mutator Phenotype

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ABSTRACT

The majority of tumors from hereditary nonpolyposis colorectal cancer families and a subset of unscreened gastrointestinal and endometrial tumors exhibit a microsatellite mutator phenotype (MMP) that leads to the accumulation of hundreds of thousands of clonal mutations in simple repeat sequences. The mutated genes with positive or negative roles in cell growth or survival are not well characterized. Here, we report that endometrial cancer of the MMP also accumulates mutations in genes that are typically mutated in gastrointestinal cancer of the mutator phenotype, including caspase-5, a member of the caspase family of proteases that has an (A)10 repeat within its coding region, in MMP tumors of the endometrium, colon, and stomach (28, 62, and 44%, respectively). We therefore suggest caspase-5 as a new target gene in the microsatellite mutator pathway for cancer.

INTRODUCTION

The MMP, also known as MSI or replication error, characterizes the majority of tumors arising in patients of the hereditary nonpolyposis colorectal cancer syndrome and a subset of randomly selected tumors of the colon, stomach, and endometrium (1–6). The MMP represents a distinct pathway for cancer development, the mutator pathway, because the genes with positive or negative roles in cell growth or survival (cancer genes) found altered in these tumors are generally different from the cancer genes that are involved in the suppressor pathway for aneuploid gastrointestinal cancer. For instance, whereas APC, K-ras, and p53 mutations are typical in colorectal cancer of the suppressor pathway (7), these genes are infrequently mutated in colon cancer of the MMP (1, 6, 8–11).

Tumors of the MMP show an enhanced, widespread genomic instability at microsatellites, resulting from the inactivation of genes of the DNA mismatch repair system (5, 6, 12). Six genes have been characterized to play functional roles in the DNA repair system: hMLH1, hPMS1, and hPMS2 (homologous to the Escherichia coli MutL DNA mismatch repair gene) and hMSH2, hMSH3, and hMSH6 (homologues to the bacterial MutS gene; Refs. 12 and 13). Hypermethylation of the hMLH1 promoter has been reported in MMP tumors (14–16), suggesting hypermethylation as a new mechanism involved in the establishment of this mutator phenotype (14).

Although microsatellites are typically long repeats, shorter repeats can be found within the coding regions of some genes. Frameshift mutations in mononucleotide tracts have been found in a subset of genes containing these repeats in MMP gastrointestinal tumors. These include the growth factor receptors TGFβRII (17) and IGFIIR (18) and the proapoptotic gene BAX (19). In addition to these cancer genes (those involved in cell growth or survival), other genes from the DNA mismatch repair family, hMSH3 and hMSH6, have been also found mutated in gastrointestinal tumors of the MMP (20).

In accordance with these findings, we proposed a model for the microsatellite mutator pathway (21), in which the first step was the mutational inactivation of the DNA repair genes hMLH1 or hMSH2 (primary mutators), followed by the inactivation of other secondary mutator genes (hMSH3 and/or hMSH6) by frameshift mutations induced by the primary mutators. In support of this model, we recently reported mutations in hMSH3, hMSH6, TGFβRII,IGFIIR, and BAX in colorectal and gastric tumors of the MMP (22, 23).

A fraction of endometrial tumors also manifest the MMP (4, 24). Mutations in the mismatch repair genes hMLH1, hMSH2, and hMSH3 have been reported in endometrial tumor cell lines (25, 26). In contrast, frameshift mutations in the TGFβRII gene, which are extremely common in colon and gastric tumors (17, 22, 23), are rare in endometrial cancer of the MMP (27). It was of interest to further examine endometrial cancer for MMP target genes to determine the similarities and differences in genotype between endometrial and gastrointestinal cancers of the microsatellite mutator pathway.

We detected common frameshift mutations in BAX, hMSH3, and hMSH6 in MMP endometrial carcinomas and endometrial tumor cell lines. We also analyzed the methylation status of the hMLH1 promoter in these endometrial tumors and found hypermethylation in 33% of them. We also report a high incidence of mutations in caspase-5 in endometrial, colon, and gastric tumors and cell lines of the MMP. Although the level of genomic instability and the incidence of mutations in target genes for the MMP are generally lower in endometrial cancer than in gastrointestinal cancer, our results show that MMP endometrial cancer also follows a similar mutator pathway and suggest caspase-5 as a new common target gene involved in this pathway.

MATERIALS AND METHODS

Tumor Specimens and Cell Lines. Endometrial tumors were obtained from the Center d’Investigacions en Biologia Molecular Vall d’Hebron (Barcelona, Spain) and the Hospital Clínico Universitario San Carlos (Madrid, Spain). The origins of the colon and stomach tumor samples were described previously (19–23). Ampullary tumors were a generous gift of Dr.
and D17S846, from the following set: D3S1611, D4S2989, D4S2939, D4S1576, D4S1611, D4S402, D4S2975, D4S411, D4S1564, D5S418, D5S421, D8S199, D9S176, D9S272, D9S1690, D17S855, 19–23) by using two mononucleotide repeats, (A)18 of ...Chomczynski and Sacchi (28).

The MMP was analyzed by PCR as described previously (1, 29) by using two mononucleotide repeats, (A)9 and (D)10 of BAT26 (29), and the dinucleotide repeat DIS158 (30). Additional microsatellite loci were analyzed in some cases (Table 1). The PCR primers for these microsatellite loci have been described (22, 23). Tumors were classified as MMP positive or negative by using the two mononucleotide repeats. The use of two mononucleotide repeats rather than one (31, 32) was necessary because of the existence of shorter BAT26 alleles that may be misdiagnosed as the typical shortening of mononucleotide repeats in tumors of the MMP, especially among African-Americans (33). The average number of deleted bp in AP2 and BAT26 for each tumor was approximated by comparing the average sizes of PCR products from the tumor with its normal tissue counterpart. When no normal DNA was available, the calculation was carried out using the size of the most common alleles (18 and 26 nucleotides, respectively).

Frameshift Mutation Analysis. PCR was carried out with Vent DNA polymerase (New England Biolabs, Beverly, MA) in the presence of 0.2 mCi of [α-32P]dCTP as follows: 1 cycle at 94°C for 4 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s. PCR products were analyzed on a 6% denaturing polyacrylamide gel and subjected to autoradiography. The corresponding PCR primers for all genes except caspase-5 have been described previously (19–23). To amplify the mononucleotide repeat within caspase-5, we sequenced 352 bp of the intronic sequence localized immediately upstream from the repeat using the available cDNA sequence information (34). Thus, the corresponding primers for caspase-5 were 5'-CAG AGT TAT-3' and 5'-CAG AGT TAT-3' for the digestion control insert (5'-CA TGT CAC AGA GGA TAA GGA GA-3' and 5'-GAC TCA AAA CAC TAG TGA GG-3') and for a 207-bp region of hMLH1 without restriction sequences for MspI or HpaII (5'-AGA GAT TTG GAA AAT GAG TAA C-3' and 5'-GAC AAT ATC ATC AGA GGA GG-3'). PCR was carried out with Taq polymerase (Perkin-Elmer Corp., Branchburg, NJ) for 1 cycle at 94°C for 4 min, followed by 35 cycles at 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s. PCR products were analyzed by electrophoresis.

RESULTS

MMP Analysis of Human Tumors.

In our accumulative analysis for the MMP of nearly 1000 primary human tumors, we found only...
positive cases among tumors from the colon, stomach, and pancreas as well as in 1 of 27 prostate cancers (Table 1). In addition, 3 of 12 ampullary tumors were MMP+. Now, we report 16% incidence (18 positive cases from 112 total tumors) of endometrial tumors displaying the MMP (Tables 1 and 2). The endometrial tumor cell lines AN3CA, RL95-2, SK-UT-1, SK-UT-1B, HEC-1A, and HEC-1B were also positive for the MMP. The MMP status of most endometrial tumors and cell lines was determined in the absence of matching normal tissue, with two mononucleotide repeats (33). BAT26 alleles shorter than \((A)_8\) were detected in the normal tissues of 1 of 153 (0.6%) Caucasians and in 8 of 59 (13.5%) African-Americans.

According to our diagnostic criteria (“Materials and Methods”), not a single MMP+ tumor was found among tumors of the lung (both adenocarcinomas and squamous cell carcinomas), breast, kidney, thyroid, liver, ovary, and esophagus (Table 1). Sporadic alterations in dinucleotide but not mononucleotide microsatellites (usually of only one repeated unit) were found in some of these tumors, which we termed MMP+/-.- However, no mutations have been found in the target genes for the MMP in these tumors (Table 1). Moreover, in a comparative study of colorectal tumors, we found that these MMP+/- tumors (also known as MSI-L, according to a National Cancer Institute Workshop on Microsatellite Instability; Ref. 35) were indistinguishable in phenotype from tumors without detectable microsatellite alterations (MMP-) in all parameters examined (Refs. 33 and 36 and data not shown).

**Frameshift Mutations in hMSH3 and hMSH6 in MMP Endometrial Cancer.** We analyzed frameshift mutations in the \((A)_8\) tract region within the hMSH3 and in the \((C)_8\) tract of the hMSH6 DNA mismatch repair genes (Fig. 1). Mutations in these repeats were detected in 28% (5 of 18) and 17% (3 of 18), respectively, of the MMP+ endometrial tumors and in 16% (1 of 6) and 66% (4 of 6) of the MMP+ endometrial tumor cell lines (Tables 1 and 2). Homozygous deletions of hMSH6 were apparent in the tumor cell lines HEC-1A and HEC-1B (Fig. 1). Endometrial tumors 83, 100, and 162 showed homozygous deletions of hMSH3, although due to the contaminating normal tissue present in the samples, a faint band corresponding to the normal allele is also seen (Fig. 1). Additional mutations in the \((A)_8\) repeat of hMSH3 were found in one prostatic adenocarcinoma, two ampullary tumors, and one pancreatic adenocarcinoma (Table 1). No mutations in the hMSH3 or hMSH6 genes were found in any MMP+ endometrial and other tumors and cell lines. No mutations were found in the MMP+ tumors in other repeats of \((A)_8\) within the hPMS2 and \((C)_8\) within the NSEP genes (Table 1).

**hMLH1 Methylation and Expression in MMP Endometrial Cancer.** Methylation of the hMLH1 promoter was found in 33% (6 of 18) of MMP+ endometrial tumors (Table 2 and Fig. 2). No hypermethylation was found in 15 MMP+ endometrial tumors or in any additional MMP+ tumors from the lung (24 cases), stomach (30 cases), or colon (35 cases; data not shown). With the exception of AN3CA and SW48, all other MMP+ and MMP− analyzed cell lines (Table 2) were negative for hMLH1 hypermethylation and thus expressed the gene (data not shown).

**BAX Frameshift Mutations in Endometrial Tumors of the MMP.** We analyzed the region encompassing the \((G)_8\) repeat within the BAX gene in the endometrium tumors and tumor cell lines (Fig. 1). About half of the tumors (10 of 18) and cell lines (3 of 6) exhibited frameshift mutations in this gene (Table 2). No mutations in the other BAX allele were detected by single-strand conformation polymorphism analysis in the endometrial tumors and tumor cell lines analyzed, nor were mutations found in other MMP− tumors and tumor cell lines of the endometrium, lung, pancreas, prostate, ovary, breast, stomach, and colon (Tables 1 and 2). No mutations were detected in the \((G)_8\) tract within the gene NGFR in any of the endometrial tumors and cell lines analyzed.

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**Table 2 Frameshift mutations in MMP+ endometrial tumors and cell lines**

<table>
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<th>MMP+ endometrial tumor cell lines</th>
<th>hMLH1&lt;sup&gt;a&lt;/sup&gt; methylation</th>
<th>hMSH3&lt;sup&gt;b&lt;/sup&gt; (A)&lt;sub&gt;8&lt;/sub&gt;</th>
<th>hMSH6&lt;sup&gt;b&lt;/sup&gt; (C)&lt;sub&gt;8&lt;/sub&gt;</th>
<th>BAX&lt;sup&gt;b&lt;/sup&gt; (G)&lt;sub&gt;8&lt;/sub&gt;</th>
<th>IGFIIb&lt;sup&gt;b&lt;/sup&gt; (G)&lt;sub&gt;8&lt;/sub&gt;</th>
<th>caspase-3&lt;sup&gt;b&lt;/sup&gt; (A)&lt;sub&gt;10&lt;/sub&gt;</th>
<th>TGFβRII&lt;sup&gt;b&lt;/sup&gt; (A)&lt;sub&gt;10&lt;/sub&gt;</th>
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<sup>a</sup> - not methylated; +, methylated.

<sup>b</sup> - wild type; +, mutant; +d, 1-bp insertion; +/u, homzygous insertions of 1 and 2 bp; +d, 1-bp deletion; +/d+d, homzygous deletion of 1 bp.

<sup>d</sup> - Hypermethylation was absent in three MMP− endometrial (Hs34.T, Hs248.T, and Hs825.T) and colon SW620 cell lines and in MMP+ colon carcinoma (LoVo, LS411N, DLD-1, LS174, LS180, and HCT116) and prostate carcinoma DU-145 cell lines. The three MMP− endometrial cell lines were negative for mutations in all the target genes. Additional MMP− cell lines were also negative for mutations, including: 16 from colon (SW620, SW403, SK-CO1, NCI-H747, NCI-H508, Caco-2, HT-29, BM314, CHC-Y1, COL0201, RC1-M, SW387, SW1417, WiDr, and COCO320DM); 9 from pancreas (MiAPaCa-2, Capan-1, Capan-2, PAN C-1, A-Pc-1, HPAF, Hs766T, BxPc3, and Hs578T); 7 from stomach (AZ521, JRST, KATOIII, MKN 1, MKN28, MKN 45, and MKN74); 2 from lung (A549 and SK-LU-1); and 1 each from small intestine (HcTu80), ovary (PA-1), and breast (MDA-MB-468).
Low Incidence of TGFβRII and IGFIIR Frameshift Mutations in MMP Endometrial Cancer. Mutations in TGFβRII and IGFIIR have been detected in MMP+ colon and gastric carcinomas (17, 18, 22, 23, 27). We analyzed the regions of these genes that encompass the (G)8 tract within IGFIIR and the (A)10 tract within TGFβRII in endometrial tumors and cell lines. Mutations in the IGFIIR (G)8 tract were detected in some of the endometrial cell lines, but no mutations were found in any of the 18 MMP+ endometrial tumors (Table 2). On the other hand, no mutations in TGFβRII were detected in the endometrial cell lines, and a low incidence of 5% (1 of 18) was found in primary tumors (Table 2 and Fig. 1). Mutations in TGFβRII were found in two of three MMP+ ampullary tumors (Table 1). No mutations in IGFIIR or TGFβRII were found in tumors and cell lines without the MMP, including those of the endometrium. No mutations were found in the (A)10 repeat within the SCP-1 gene in any of the MMP+ endometrial tumors and cell lines.

Frameshift Mutations in caspase-5 in MMP Cancers. The low incidence of frameshift mutations in the TGFβRII gene in endometrial cancer was in contrast to the high frequency of mutations found in MMP colon (82%) and stomach (72%) tumors (22). This suggested that other genes may be mutated in endometrial cancer of the MMP. A search for genes with mononucleotide tracts in their coding regions yielded a (A)10 tract in the caspase-5 gene (34). In contrast to the near absence of mutations in the (A)10 tract of TGFβRII in endometrial cancer, we detected a relatively high incidence of mutations in the caspase-5 identical repeat. Tumor cell lines AN3CA, RL95–2, SK-UT-1, and SK-UT-1B and 28% (5 of 18) of MMP endometrial tumors showed 1-bp deletions (Tables 1 and 2 and Fig. 1). However, mutations in caspase-5 were not endometrial cancer specific because they were also found in other MMP colon and stomach tumors (Fig. 3).

These mutations did not appear to be generated during establishment in vitro because their incidence was higher in primary tumors than in tumor cell lines. Thus, 62% (24 of 39) and 44% (11 of 25) of colon and stomach MMP tumors were positive for frameshift mutations in this gene (Table 1 and Fig. 3). Among tumor cell lines, DLD-1 showed a 1-bp insertion, whereas LS174T and LS180 showed a homozygous (or hemizygous) 1-bp deletion. Insertions of 1 bp were found in some gastrointestinal tumors, for instance, colon tumor 201 and stomach tumors J28 and J64T (Fig. 3). Insertions and deletions of 2 bp (but not of 3 bp) were also found (data not shown and Fig. 4). No caspase-5 frameshift mutations were found in cell lines and gastrointestinal tumors without the MMP (Tables 1 and 2). Sequence analysis of some representative caspase-5 selected mutated sequences demonstrated insertion and deletion mutations in the (A)10 tract (Fig. 4).

Comparative Analysis of Mononucleotide Repeat Mutations in Tumors of the MMP. A comparative PCR analysis of mononucleotide repeats revealed that the average number of deleted nucleotides of BAT26 was 5 ± 1 for endometrial tumors, whereas those for colon and stomach tumors were 9 ± 2 and 11 ± 3, respectively (Fig. 5). The average deletions for APA2Δ were 2 ± 1 for endometrial tumors and 5 ± 1 and 6 ± 1 for stomach and colon tumors, respectively (Fig. 5). We also compared the incidence of mutations in MMP target genes according to the number of mutated alleles (Fig. 5). The percentage of mutations in endometrial tumors was lower than in gastrointestinal tumors for each one of the genes analyzed. On the other hand, colon tumors showed a lower incidence of mutations in hMSH3, hMSH6, and BAX and a higher incidence of caspase-5 and TGFβRII mutations relative to stomach tumors. Because mutations in mononucleotide repeats are always deletions in MMP+ tumors (1, 30–33), these...
mutations may be used as molecular clocks that reflect the number of cell replications undergone by the corresponding tumor cells (30, 37). Therefore, it appears that endometrial tumors accumulate fewer microsatellite mutations than gastrointestinal tumors. As a corollary, endometrial tumors appear to have sustained less number of cell replications since the manifestation of the MMP.

DISCUSSION

Tumors of the MMP accumulate hundreds of thousands of mutations (deletions of a few nucleotides) in mononucleotide repeats throughout the genome (1, 38). Detection of deletion mutations in these mononucleotide repeats is, therefore, diagnostic of widespread genomic damage with functional significance for cancer development. Clonal alterations in mononucleotide repeats are restricted to gastrointestinal and other cancers associated to the hereditary nonpolyposis colorectal cancer syndrome and are not found in the absence of a functional mutator phenotype due to, for instance, impaired DNA mismatch repair activity. In contrast to these somatic contractions in neutral (noncoding) mononucleotide repeats, alterations in other microsatellites of two, three, and four nucleotides can be also found with a low incidence in the majority of human tumors (2, 3, 39, 40).

The generalized use of dinucleotide repeats has lead to the classification of many tumors as positive for MSI, implying a genomic instability that plays a functional role in cancer progression. However, there is no experimental evidence to support that tumors with this low level of MSI at dinucleotide (or tri- or tetranucleotide) repeats (35) have inherited or acquired deficiencies in any of the known genes implicated in the preservation of the replication fidelity of these unstable sequences (33). Although these microsatellite alterations may be useful markers of clonality (39) or of mitotic activity (30, 37), they are not necessarily diagnostic of genomic instability. We concluded (19, 22, 23, 33) that these sporadic somatic alterations in dinucleotide (or tri- or tetranucleotide) repeats are the result of their relatively high endogenous mutation rate. We also report here that none of these MMP+/- tumors have mutations in the target genes for the MMP (Table 1). Thus, for all practical purposes related to genomic instability and the corresponding diagnostic applications for cancer and cancer susceptibility, these MMP+/- or MSI-L tumors (35) can be considered as tumors without detectable microsatellite alterations.

Although the incidence of mutations in TGFβRII and IGFIIR in endometrial tumors is very low, compared to colon and gastric cancers, the incidence of BAX mutations remains high; hence, mutations in BAX also appear to be under positive selective pressure during endometrial tumorigenesis. This conclusion is reinforced by the fact that no mutations were detected in repeats of (G)₈ within other genes such as NGFR and IGFIIR.

Another question we approached was whether or not MMP endometrial tumors also follow the model of the “mutator that mutates another mutator” (21). As we recently reported (20–23), mutations in the hMSH3 and hMSH6 repair genes are frequent in MMP tumors of the gastrointestinal tract, some of which contain mutations in primary mutators such as hMLH1. Methylation of the hMLH1 promoter that has been found associated with lack of hMLH1 expression in colorectal tumors (14) also may play a role in endometrial cancer of the MMP. Whether this hypermethylated status extends to other mismatch repair genes, such as hMSH3 and hMSH6, is currently under investigation. Here, we report mutations of the hMSH3 and hMSH6 genes in MMP endometrial tumors and cell lines, although the incidence of these mutations is lower than in MMP colorectal and gastric cancers (Table 1). This is consistent with the lower extent of microsatellite alterations found in endometrial tumors (Fig. 5). However, according to the mechanism of slippage during replication (41), mutations in the (A)₁₀ repeat within TGFβRII should be more frequent than slippage errors in shorter repeats like the (A)₈ within hMSH3. Hence, the presence of common mutations in the hMSH3 gene is more likely to be relevant. Moreover, mutations were not detected in another (A)₁₀ repeat within the SCP-1 gene. These results contrast with the higher incidence of mutations detected in the (A)₁₀ repeat of caspase-5.
Fig. 5. Incidence of mutations in mononucleotide repeats in MMP endometrial and gastrointestinal tumors. The graph represents the percentage of mutations expressed by the percentage number of mutated alleles versus the total number of alleles. Genes are named at the bottom, and the allelic percentage of positive cases are written above each bar. The (A)_{15} (BAT26) and (A)_{18} (APD2) columns represent the average number of bp deletions found in the tumors, expressed as a percentage and using 100 as the average number of deletions of BAT26 in colon tumors. The calculations are only approximate because of the difficulties in determining the exact number of deleted bp in the noncoding repeats, especially when no normal tissue was available (see “Materials and Methods”), and whether the frameshift mutations in target genes were homozygous or heterozygous (see Figs. 1 and 3). The estimation of the number of deleted bp in noncoding repeats was done by comparing the sizes of the smaller of the PCR bands amplified from tumor and normal tissue DNAs.

Mutations in this gene were also found in other gastrointestinal tumors and cell lines of the MMP, suggesting that mutations in caspase-5 are under positive selection pressure in cancer of the MMP irrespective of the tissue of origin. In addition to the absence or lower incidence of frameshift mutations from other identical repeats in other genes, including TGFBRII, the detection of insertions mutations in some tumors argue in favor of a relevant role of these mutations in cancer progression. This reasoning is based on the observation that in MMP tumors the mutations found in neutral microsatellite (A)_{n} sequences have been deletions without exception (1, 31–33). Insertions in (A)_{n} tracts have been found only in genes that, when inactivated, may lead to alterations in cell growth, such as the TGFBRII (17, 22, 23, 27) or the APC tumor suppressor gene (42). Furthermore, although deletions of one nucleotide are common in the (A)_{n} tract of the hMSH3 gene, therefore reducing the tract length from 8 to 7 bp [(A)_{8}→(A)_{7}], we never found deletions of three bp in TGFBRII or caspase-5 ([A]_{10}→[A]_{7}) in cancers of the MMP; this suggests that in-frame mutations in these genes do not exhibit a selective advantage. Consequently, these results reinforce the relevance of the presence of mutations that lead to frameshifts.

Nevertheless, we cannot rule out that the caspase-5 mutations found in these tumors may have no functional role in tumorigenesis because of the maintenance of the ratios in these three types of tumors between the relative frequency of mutations in this gene with the relative frequencies of neutral microsatellite sequences (Fig. 5). On the other hand, the alterations in the ratios of mutations in hMSH6, hMSH3, and BAX genes between endometrium, gastric, and colon cancers, although statistically not significant, suggest that at least some of these mutations are relevant because they imply that they are under differential selective pressures in these tumors.

Assuming that the caspase-5 frameshift mutations are functional, the precise role that they play in tumorigenesis remains unknown because the function of the gene is also unknown. Proteases of the caspase family are related both to programmed cell death and to immune and inflammatory responses. caspase-4 and caspase-5 are closely related to caspase-1 and constitute the caspase-1-like subfamily (43). Like caspase-1, caspase-4 and caspase-5 both cleave pro-caspase-3 to its active form, caspase-3 (44). Further, caspase-4 and caspase-3 are processed after Fas stimulation and can trigger the cell to apoptosis (45). However, caspase-1 encodes a protein resembling ICE (46) that cleaves pro-interleukin 1β to interleukin 1β (47) and IFN-γ-inducing factor (48). ICE has been shown to be also an important regulator of immune and inflammatory response and inflammation could be a major cause of cell death (49, 50). Accordingly, inhibition of either ICE-apoptotic pathway or inflammatory response could be involved in tumor progression. Whether the selective advantage of cells with caspase-5 mutations in MMP tumors is due to inhibition of apoptosis or to inhibition of the inflammatory response remains to be clarified. Homozygous deletions of caspase-5 were found in colon tumor cell lines LS180 and LS174T (two phenotype-different clones but derived from the same original tumor; see Ref. 30). These cell lines should be useful tools to study the effects of caspase-5 inactivation in tumorigenesis and apoptosis.

The homozygosity of a tumor-linked frameshift mutation is difficult to establish in primary tumor specimens due to the contaminating normal tissue. In certain cases (tumors 83, 100, and 162) the deletions of hMSH3, for instance, appeared homozygous. But, overall, our results indicate that many of the endometrial tumor cells with mutations in caspase-5, BAX, hMSH3, and hMSH6 genes were heterozygous for these mutations. The biological significance of these heterozygous mutations poses an intriguing question because of the recessive nature of the MMP (51). The presence of other inactivating mutations in the other allele was found in some MMP colon and gastric tumors by analyzing both alleles of BAX (22). However, at this point, no mutations have been detected in the other allele of BAX in any tumors of our endometrial collection. These results lead to the conclusion that heterozygous mutations in BAX may be sufficient to ensure their clonal perpetuation. This point has already been suggested by other authors who found cellular resistance to apoptosis in heterozygous knockout mice for BAX (52). The search for mutations in other alleles of caspase-5, hMSH3, and hMSH6 are under current investigation; we already detected another inactivating somatic mutation in hMSH6 in the MMP endometrial tumor sample 153, which also has a 1-bp deletion in the (C)_{n} tract of the other allele.

Due to the exacerbated mutator phenotype of these tumors, their malignant phenotypes may have resulted from the progressive accumulation of alterations on the expression of several genes playing specific roles in various cell growth, apoptotic, or repair pathways.
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Although, in tumors without the mutator phenotype, the contribution to the cancer phenotype is usually accomplished by the biallelic mutation of a rate-limiting gene (i.e., APC), in tumors of the MMR, multiple heterozygous mutations in various genes located at different levels of the same signaling pathways may also result in the collapse of the equilibrium required for normal cell growth, cell survival, or DNA repair, associated with neoplastic transformation or with the mutator phenotype that indirectly leads to this transformation.

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