Microsatellite Instability in Colorectal Cancer: Different Mutator Phenotypes and the Principal Involvement of hMLH1


Departments of Laboratory Medicine and Pathology [S. N. T., A. J. F., J. M. C., D. T., L. J. B., P. C. R.], Health Sciences Research, Section of Biostatistics [S. K. M., D. J. S.], Medical Genetics [C. W. V., V. V. M.], and Oncology [M. J. O.], Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55905, and Ochsner Clinic, New Orleans, Louisiana 70121 [G. H. F.]

ABSTRACT

Recent studies have demonstrated the presence of microsatellite instability (MSI) in tumors from patients with hereditary nonpolyposis colorectal cancer and in a large number of sporadic tumors. To further characterize the type of alterations at these loci and their frequency of involvement in colon cancer, we studied DNA extracted from paraffin-embedded tissue from 508 patients using 11 microsatellites localized to chromosomes 5, 8, 15, 17, and 18. Overall, MSI at each locus varied in character and frequency and was observed with at least one marker in 191 cases (37.6%). Based on the number of markers displaying instability per tumor, three groups of patients were defined: those with <30% of the markers showing instability (MSI-L, n = 109, 21.5%); those with ≥30% (MSI-H, n = 82, 16.1%); and those showing no instability (MSS, n = 317, 62.4%). These groups were tested for correlations with a number of clinical and pathological parameters, including age, sex, stage, ploidy status, and site of tumor. Comparing across the three groups and verified by pair-wise comparisons, the MSI-H group was associated with tumor site (proximal colon, P = 0.001), sex (females, P = 0.005), stage (Dukes’ B, P = 0.01), and ploidy status (diploid, P = 0.03). No significant differences were noted between the MSI-L and MSS group for any of the parameters tested. An additional 188 consecutive surgical colorectal cancer cases were examined for the presence of MSI and for the immunohistochemical expression of hMLH1 and hMSH2 proteins. Of this group, 129 (68.6%) were classified as MSS, 17 (9.0%) as MSI-L, and 42 (22.3%) as MSI-H. None of the MSS and none of the MSI-L tumors had altered expression of either hMLH1 or hMSH2. However, the majority of MSI-H (40 of 42, 95%) cases demonstrated absence of staining for these proteins. The most frequently altered protein was hMLH1, occurring in 95% of the tumors with altered expression. Cumulatively, these data suggest that the tumor phenotype MSI-H is distinct from tumor phenotypes MSI-L and MSS, with no apparent differences between MSI-L and MSS. Furthermore, altered hMLH1 protein expression appears to be responsible for the mutator phenotype in the vast majority of MSI-H tumors.

INTRODUCTION

Microsatellites are tandem repeats of simple sequences that occur abundantly and randomly throughout the human genome. They consist of ~10–50 copies of 1–6 bp motifs that can occur as perfect tandem repeats, as imperfect (interrupted) repeats or as a combination of the two repeat types (1). The length of a given repeat sequence is often polymorphic and is normally transmitted through mitosis and meiosis without alteration. Although microsatellites are not known to have any specific function, they have been postulated to act as promoters, sites of recombination, or binding sites for DNA topoisomerasers (2–4).

Recent studies have demonstrated novel alterations of microsatellite DNA in tumor tissue (5–7). These alterations, termed MSI-H, have been observed in tumors from patients with HNPPC (7–9), the Muir-Torre syndrome (10, 11), and in a subset of patients with sporadic colorectal cancer (5–8, 12–15). These observations, along with the results of genetic linkage analysis in HNPPC (16, 17) and the elucidation of DNA mismatch repair mechanisms in bacteria and yeast (18, 19), led to the identification of two major genetic susceptibility loci for HNPPC, hMSH2 and hMLH1. These two genes, as well as hMSH3, hMSH6, and hPMS2, all participate in DNA mismatch repair (reviewed in Refs. 20 and 21). Although defective mismatch repair is believed to be responsible for the MSI phenotype in the majority of tumors from patients with HNPPC, the genetic defect responsible for this phenotype in sporadic colon cancer has yet to be clearly delineated. Somatic and germ-line alterations in both hMSH2 and hMLH1 have been identified in some cases, but not all (15, 22–26). Overall, the rate of involvement for these genes in sporadic colorectal cancer with MSI has been lower than anticipated. Additionally, the relative involvement of either hMSH2 or hMLH1 in these sporadic colorectal cancers is unknown.

MSI is not unique to tumors of the colon. The presence of MSI has been reported in a variety of malignancies, including cancer of the endometrium, stomach, pancreaticobiliary system, ovary, prostate, breast, lung, and renal pelvis and ureters (27). As is the case for colon cancer, however, MSI in other neoplasms is thought to be nonspecific and secondary to possible common pathways, such as defective mismatch repair. The underlying molecular basis for the MSI in many of these other tumor systems, however, has not been clearly established.

In spite of the multitude of published studies to date, there is still confusion as to how one defines tumor MSI. That is, how many markers should be used, which markers should be used, and how many markers must display instability before a tumor is defined as having MSI. Because of differences in criteria used to define the MSI tumor phenotype and differences in both marker selection and the total number of markers used, a comparison of various studies has been difficult. Such nonuniformity has led, in part, to the considerable variability in the frequency of instability reported both within and between different tumor types.

In this study, a number of microsatellite loci were analyzed in colorectal cancer to better define both the types of alterations and their frequency of involvement in colorectal cancer to determine whether there are different mutator phenotypes and to explore their association

3 The abbreviations used are: MSI, microsatellite instability; MSI-L, low-frequency MSI; MSI-H, high-frequency MSI; MSS, microsatellite stable; HNPPC, hereditary nonpolyposis colorectal cancer; NCCTG, North Central Cancer Treatment Group; OR, odds ratio; CI, confidence interval.

with a number of clinical and pathological features. Tumors were also examined immunohistochemically to determine the relationship between tumor MSI and the involvement of the DNA mismatch repair proteins hMLH1 and hMSH2 in unselected colorectal cancer. Results from these studies indicate the presence of distinct MSI-related phenotypes that differ with respect to clinical and pathological parameters, as well as their underlying mechanistic processes.

MATERIALS AND METHODS

Patient Population. Paraffin-embedded tumor and normal tissue was obtained from two groups of patients with colorectal cancer. The first group \((n = 508)\) was selected from those patients undergoing adjuvant chemotherapy through the NCCTG. For these patients, tissue was obtained at surgery prior to chemotherapy. Of the 508 patients, 286 were male and 222 were female, with the age at diagnosis ranging from 25 to 88 years. Of the 508 tumors, 222 were from the proximal colon, and 286 were from the distal colon; 181 were classified as Dukes’ B, and 327 were classified as Dukes’ C. For the immunohistochemical analysis, tumor and normal tissue was obtained from a second group of patients \((n = 188)\) participating in a prospective cancer risk assessment study. This latter group of patients were consecutive surgical cases who consented to participate in this study and were comprised of 111 males and 77 females, ranging in age from 18 to 89 years.

Patients with familial polyposis were excluded from this study. Other than familial polyposis, family history of colon cancer was not considered, and thus, it is likely that some cases of HNPCC may be present.

Marker Selection. For the first group of 508 normal/tumor pairs, 11 microsatellite markers were used to test for MSI. These included D5S346 and DSS107 localized near the APC gene on 5q, D8S254 located near a putative tumor suppressor gene on 8p, ACTC on 15q, D17S261 and TP53 on 17p at or near the P53 locus, and D18S34, D18S49, D18S35, D18S61, and D18S58 localized on 18q both proximal and distal to DCC. Each of these markers are dinucleotide repeats. DNA from tumors in the immunohistochemistry study were tested with six of the markers used above \((D5S346, TP53, D18S61, D18S34, D18S49, and ACTC)\) plus one additional mononucleotide repeat \((BAT 26)\).

DNA Extraction. DNA was extracted from both normal and malignant tissue specimens that were processed conventionally by formalin treatment and paraffin embedding. Tissue was cut into 10-μm-thick sections and then mounted onto glass slides. One reference slide was stained with H&E, whereas two others were stained with toluidine blue. Areas of normal and tumor were identified and marked on the toluidine blue-stained slides; areas of tumor contained >70% cancer cells. The tissue was then scraped into microcentrifuge tubes, and the DNA was extracted with one of two procedures. For the first, 100 μl of digestion buffer \((50 \text{ mm Tris, pH 8.0)}\), together with 1 μl of proteinase K \((20 \text{ mg/ml})\), was added to ~1 cm² of tissue. The samples were then incubated at 55°C for 48 h, with an equal volume of proteinase K added after the first 24 h. The resulting digest was subjected to centrifugation to remove any remnant undigested tissue, boiled for 8 min at 95°C to inactivate the proteinase K, placed in an ice-water slurry for 5 min, and finally stored at 4°C until needed for analysis. For the second procedure, DNA was extracted using the Qiamp Tissue kit \((Qiagen, Inc., Santa Clarita, CA)\) according to the manufacturer’s instructions. Both procedures provided comparable results.

PCR Analysis and Interpretation. The PCRs and gel electrophoresis conditions were essentially as described previously \((5)\). Although all 11 markers were used on each of the normal/tumor pairs, there were some PCR failures due to the degraded nature of the paraffin-embedded tissue. This was primarily the case for the NCCTG cohort of patients. On average, however, at least eight markers were successfully amplified, and tumors were scored for instability only if at least 5 of the 11 markers successfully amplified for both the normal and tumor DNA. For the immunohistochemical portion of the study, a minimum of five markers were used to score for MSI. In the majority of cases, all seven markers used were informative for MSI.

Immunohistochemical Analysis. Immunohistochemical staining was performed on 6-μm-thick, formalin-fixed, paraffin-embedded tissue sections. Sections were deparaffinized and rehydrated through graded alcohols to water. Endogenous peroxidase activity was blocked by incubation with 0.6% H₂O₂, Sections were then immersed in 10 mm sodium citrate buffer, pH 6.0, and subjected to heat-induced antigen retrieval. After treatment with 10% normal goat serum for 10 min to block nonspecific protein binding, monoclonal hMLH1 \((\text{clone G168 728; PharMingen; 1 mg/ml})\) and hMSH2 antibodies \((\text{clone FEI1; 0.5 mg/ml; Oncogene Science})\) were applied. For hMLH1 staining, tissue sections were incubated overnight at 4°C, and a labeled streptavidin-biotin method was used. After brief rinsing, sections were treated with biotinylated anti-mouse IgG for 30 min at room temperature, rinsed, and then incubated with peroxidase-labeled streptavidin for 30 min at room temperature. After brief washing, sections were incubated with diaminobenzidine and H₂O₂ for 5 min. Sections were then lightly counterstained with hematoxylin, dehydrated in graded alcohols, cleared in xylene, and coverslipped. For hMSH2 staining, tissue sections were incubated for 20 min at room temperature. After brief rinsing, the Catalyzed Signal Amplification system \((\text{DAKO Corp.)})\) was used, according to the manufacturer’s instructions, for visualization of specific hMSH2 staining. Sections were counterstained and mounted as described for hMLH1. The normal staining pattern for both hMLH1 and hMSH2 was nuclear. Tumor cells that exhibited an absence of nuclear staining, in the presence of nonneoplastic cells with nuclear staining, were considered to have an abnormal pattern. Clone FEI1 is a mouse monoclonal antibody generated with a COOH-terminal fragment of the hMSH2 protein, whereas clone G168-728 was prepared with full-length hMLH1 protein.

DNA Ploidy Analysis. DNA ploidy analysis was performed as described previously \((28)\). Of the 508 tumors from the NCCTG cohort, ploidy results were available on 215. Those cases having ploidy studies were nearly evenly distributed among the three comparison groups: 39% \((123 \text{ of 317})\) for MSS, 49% \((54 \text{ of 109})\) for MSI-L; and 46% \((38 \text{ of 82})\) for MSI-H.

Statistical Analysis. Each of the MSI groups \((\text{MSI-L, MSI-H, and MSS; see “Results” for definition})\) was assessed for associations with the following clinical and pathological parameters: patient age at diagnosis; gender of the patient; site of the tumor (proximal or distal relative to splenic flexure); Dukes’ stage of the tumor; and DNA ploidy status. Associations with categorical variables were assessed using a contingency table χ² statistics, with the exception of pathological stage, which was assessed by the Mantel-Haenszel χ² test for linear trend \((29)\). The distribution of age at diagnosis was compared using the Wilcoxon rank sum test \((30)\). Logistic regression was used to investigate the independent effects of clinical and pathological characteristics. Analyses were performed using the SAS software \((31)\). Pairwise tests of MSI \((\text{MSI-L versus MSI-H, MSI-L versus MSS, and MSI-H versus MSS})\) were corrected for multiple testing using a Bonferroni correction.

RESULTS

Pattern and Frequency of MSI. Of the initial 508 tumors evaluated for MSI, 191 \((37.6\%)\) demonstrated MSI at one or more loci. When the gel electrophoretic banding patterns were analyzed, different patterns of microsatellite alterations were observed. For the purpose of subsequent analyses, two general types of banding patterns were defined. One type, which we have called pattern 1, is seen as a ladder-like expansion or contraction of the microsatellite repeat unit \((\text{Fig. 1})\). The second type of alteration, which we have called pattern 2, is seen as a minor alteration, typically a single-repeat change above or below the expected allele fragment size \((\text{Fig. 1})\). Although pattern 1 changes were the most frequent, accounting for 71% of the changes observed, the pattern of instability appeared to be marker dependent. For example, D8S254 demonstrated almost exclusively pattern 2 changes.

Of the 191 tumors that demonstrated MSI, the number of markers showing instability for any given tumor ranged between 1 and 100% \((\text{Fig. 2})\). For example, between 1 and 10% of the markers displayed MSI in 50 tumors, whereas 90–100% of the markers demonstrated MSI in 20 other tumors. Of interest, the frequency distribution appeared to be bimodal, with some tumors demonstrating low levels of instability, whereas others demonstrated high levels of instability. For the purpose of further data analyses, two groups of tumors with MSI were defined: those tumors \((n = 109)\) with <30% of the markers demonstrating instability \((\text{MSI-L})\) and those \((n = 82)\) with 30% or
more of the markers demonstrating instability (MSI-H). Those tumors lacking MSI were defined as MSS.

The frequency and pattern of MSI was then examined for each of the 11 markers in both the MSI-L and MSI-H groups (Fig. 3). Overall, the frequency of instability per tumor for each of the 11 markers varied considerably, ranging from about 50–95% in the MSI-H groups. The predominant pattern of instability displayed by each of the markers used also varied between the two groups. Although both patterns were present in the MSI-L and MSI-H groups, pattern 1 changes were the predominant alterations seen in the MSI-H group, accounting for ~80% of the total changes, whereas pattern 2 was the predominate pattern in the MSI-L group, representing >70% of the total changes (Fig. 3).

**Clinicopathological Correlations.** MSI-L and MSI-H tumor phenotypes were then tested for possible associations with a number of clinical and pathological parameters including tumor site, gender, stage, ploidy status, and age (Table 1). When the three groups were compared, the MSI-H tumor phenotype was found to be associated with tumors occurring in the proximal colon \( (P = 0.001) \) and those occurring in females \( (P = 0.005) \). MSI-H phenotypes were also found to be associated with a lower Dukes’ stage \( (P = 0.01) \) and with tumor DNA diploidy \( (P = 0.03) \). Although not statistically significant, patients with the MSI-H phenotype tended to have an older median age of diagnosis \( (P = 0.06) \). The \( P \)s shown in table 1 are derived from the pair-wise comparisons and were corrected for multiple testing using a Bonferroni correction. To further assess the independent association of MSI-H versus MSS with clinical and pathological characteristics, stepwise logistic regression models were fit with sex, Dukes’ stage, site, and age (below the median versus above the median) as covariates. The final model included sex \( (P = 0.006; OR, 0.46; 95\% CI, 0.27–0.80) \), site \( (P = 0.0001; OR, 0.10; 95\% CI, 0.06–0.20) \), and stage \( (P = 0.005; OR, 0.45; 95\% CI, 0.26–0.78) \), demonstrating that each of these covariates was independently associated with the MSI-H status. Because of missing data, DNA ploidy status was not included in the logistic models.

No associations were observed between the MSI-L tumor pheno-
type and tumor site, gender, stage, ploidy status, or age of diagnosis. Overall, the MSI-L group had similar characteristics when compared with that of the MSS group (Table 1).

**Immunohistochemical Analysis.** In an effort to define the underlying cause of the MSI-L and MSI-H mutator phenotypes, the level of protein expression for hMLH1 and hMSH2 was examined in paraffin-embedded tissue from 188 consecutive surgical cases of colorectal cancer (Table 2). This particular group of patients was chosen to examine the relative level of involvement of either hMSH2 or hMLH1 in an unselected series of cancer cases. Additionally, at the time the immunohistochemical analyses were performed, tissue blocks were unavailable from the retrospective group of patients. In 17 (9.0%) cases, instability was observed in <30% of the markers and classified as MSI-L. In 42 (22.3%) cases, instability was observed in 30% or more of the markers and classified as MSI-H. There were 129 (68.6%) cases that did not demonstrate instability, and these were classified as MSS. All of the MSS tumors (n = 129) and all of the MSI-L (n = 17) showed normal expression of both hMLH1 and hMSH2 protein. Of the MSI-H tumors, 90% (38 of 42) showed an absence of the hMLH1 with normal expression of hMSH2, 5% (2 of 42) showed an absence of hMSH2 with normal expression of hMLH1. Only two of the MSI-H cases demonstrated normal expression of both hMLH1 and hMSH2. Interestingly, of those cases showing abnormal protein expression, hMLH1 was involved in the majority of MSI cases (38 of 40, 95%).

The presence of MSI in this group of latter tumors was also tested for associations with anatomical site and gender (ploidy results not available). As before, the MSI-H but not the MSI-L tumor phenotype was associated with tumors occurring in the proximal colon (P = 0.0001, pair-wise comparison) and those occurring in females (P = 0.05, pair-wise comparison).

**DISCUSSION.**

MSI, as it relates to neoplastic disorders, is a phenomenon widespread throughout the human genome. It involves either the insertion or deletion of one or more copies of the repetitive unit (e.g., mononucleotide repeat) within the microsatellite being examined. In PCR-based assays, instability can be detected when comparing DNA derived from tumor to that derived from normal tissue. It can be seen as either a novel discrete band in tumor DNA not observed in the corresponding normal DNA (which we have called pattern 2) or as a marked alteration in repeat length, often heterogeneous in nature and appearing as a ladder, which we have called pattern 1 (Fig. 1). The pattern of instability and the frequency that a given microsatellite will display instability appears to be marker dependent. For example, some microsatellites are very susceptible to instability (e.g., D18S34) and show predominantly pattern 1 changes, whereas others are characterized by a low level of instability (e.g., D8S254) and show predominantly pattern 2 changes. The likelihood that a microsatellite will be susceptible to instability may relate to the inherent mutation rate at that locus. Studies have shown variability in the mutation rates of di-, tri-, and tetrancleotide repeats in CEPH families (32), and we have observed that monomorphic microsatellites tend not to show instability. Furthermore, Steiner et al. (33) have identified markers that demonstrate very high rates of instability. Although these markers have proven to be very useful as markers of clonality and for the detection of minimal residual disease, they may overestimate the presence of MSI resulting from certain causes, such as defective mismatch repair. Ultimately, differences in marker susceptibility to instability could lead to differences in the number of tumors that are scored as positive for MSI. In support of this, a very recent report, which examined the frequency of MSI at multiple loci, indicated that marker selection has a significant effect on the determination of the MSI status (34, 35). Consequently, although a large number of microsatellites are available for use in such studies, it will be important to assess their analytical characteristics prior to using them in assays for detecting tumor MSI.

The mononucleotide repeat BAT 26 has been reported previously (35-37) to be an extremely specific and sensitive marker for the identification of colorectal tumors with a high frequency of MSI (defined in the manuscript as MSI-H). In this study, BAT 26 was used as one of the markers in the prospective series of patients (n = 188). In this series, none of the MSS and only 1 of 15 MSI-L cases demonstrated instability at this locus. On the other hand, all but one of the MSI-H cases tested (40 of 41) demonstrated instability. These data confirm the usefulness of this marker in distinguishing the MSS/MSI-L cases from those cases defined as MSI-H.

In addition to marker susceptibility to instability, the number of markers that demonstrate instability is also an important consideration. That is, how many microsatellites must be altered before a tumor is defined as having MSI? The answer to this question, however, may ultimately depend on the purpose of identifying MSI cases, as well as understanding the underlying cause or causes of the MSI. For the purposes of this study, MSI was defined as any level of instability. However, when the frequency distribution of the tumors with MSI was examined, a bimodal distribution was observed (Fig. 2). Based on the frequency distribution, a 30% cutoff was used to delineate two discrete populations of tumors: MSI-L and MSI-H. Other studies (35, 38, 39) have also shown the presence of a bimodal distribution in colon tumors with MSI. These studies placed their cutoff at either 30% (39), 40% (35), or 50% (38).

The likelihood that at least one marker will exhibit instability in a given tumor increases as the total number of markers used increases. Additionally, we and Dietmaier et al. (35) have shown that susceptibility to instability varies from marker to marker. Given that differences in tumor phenotype exist for colorectal cancer with MSI (i.e., MSI-L and MSI-H), then the specific markers chosen and the number of altered markers required to score a tumor for MSI is of critical importance. Data from this study suggest that the absolute number of markers exhibiting instability may not be as important as defining the overall frequency of unstable microsatellites. For colon cancer, approximately six markers should be adequate to test for tumor MSI. Additionally, if a larger number of markers are used, we suggest that a cutoff of ~30-40% be used to define two different groups of MSI tumors, only one of which (MSI-H) appears to result from defective mismatch repair.

Classified as either MSS, MSI-L, or MSI-H, a number of clinical and pathological parameters were examined for possible associations (Table 1). MSI-H tumors, which are characterized by widespread (~30%) MSI, were found predominantly in the proximal colon, occurred more frequently in females, tended to be of lower stage, and tended to be diploid. Similar associations have been observed in previous studies when tumor MSI has been defined as high-frequency instability (5, 12, 39, 40). For the MSI-L tumors, on the other hand, no statistical differences were noted when compared with the MSS group. These data suggest that MSI-L and MSI-H tumors represent distinct mutator phenotypes with potentially different molecular etiologies.

### Table 2 Mismatch repair protein expression in the different MSI phenotypes

<table>
<thead>
<tr>
<th>MSI Phenotype</th>
<th>hMLH1 (%)</th>
<th>hMSH2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSS (n = 129)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MSI-L (n = 17)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MSI-H (n = 42)</td>
<td>38 (90.5%)</td>
<td>2 (4.8%)</td>
</tr>
</tbody>
</table>
Additional support for the existence of distinct mutator phenotypes (i.e., MSS, MSI-L, and MSI-H) comes from several histopathological studies comparing colorectal tumors with and without MSI (40–43). In one study, MSI-H carcinomas were characterized by a high incidence of cribriform/solid or signet ring histology, which were uncommon in both MSI-L and MSS cases. Similarly, tumor infiltrating lymphocytes were more prominent in MSI-H compared with either MSI-L or MSS cases, whereas extracellular mucin overproduction or Crohn’s-like inflammatory infiltrates were variably present in MSI-H tumors. Kim et al. (40), Risio et al. (41), and Rüschoff et al. (42) all reported similar distinct pathology of colorectal tumors with MSI. In these particular studies, tumors were classified as having MSI if at least two of five (40), two of four (41), or two of seven (42) markers demonstrated instability (this level of instability is analogous to the MSI-H subgroup of tumors defined in this study).

To define the etiology of MSI in sporadic colon cancer and to see whether additional differences existed between MSI-L and MSI-H tumors, levels of hMSH2 and hMLH1 protein expression (as measured immunohistochemically) were examined in a consecutive series of unselected cases of colorectal cancer. The use of such a patient population would also allow us to examine the relative involvement of either hMSH2 or hMLH1 in a largely sporadic group of patients. Overall, none of the MSI-L and none of the MSS cases showed a loss of protein expression for either hMSH2 or hMLH1. In contrast, nearly all of the MSI-H cases (40 of 42) had loss of either hMSH2 or hMLH1. In addition to further supporting the notion that the two MSI phenotypes (MSI-L and MSI-H) differ in character, these data also indicate that defective mismatch repair is primarily responsible for the MSI-H phenotype. Of particular interest, the vast majority of the cases (95%) were due to an absence of hMLH1. The involvement of hMSH2 in these cases appears to be minimal. In a recent study, Dietmaier et al. (35) also examined the expression of hMLH1 and hMSH2 in high MSI and low MSI colorectal cancers. These investigators also found altered expression to be restricted to MSI-H cancers, but in this case, both hMLH1 and hMSH2 were equally involved. Differences in the frequency of hMLH1 involvement between the two studies may be due to differences in the patient population or the number of cases having HNPCC or a familial component.

Current data suggest that germ-line mutations in either hMSH2 or hMLH1 occur in roughly equal proportions in HNPCC kindreds and between the two account for 60–70% of HNPCC mutations (44, 45). Why hMLH1 is preferentially involved in MSI-H sporadic colorectal cancer (in this study), however, is not known at this time but may indicate that this particular gene is more susceptible to somatic alterations. Herfarth et al. (46) reported that mutations in hMLH1 are more frequent than in hMSH2 in sporadic colorectal cancers with MSI, which is consistent with our observation of a preferential involvement of hMLH1 in MSI-H sporadic colorectal cancers. In general, however, the frequency of either hMLH1 or hMSH2 mutations in sporadic colorectal cancers with MSI has been less frequent than anticipated. These data, therefore, suggest that a mechanism other than mutation may be affecting gene function in these tumors. One potential mechanism for gene inactivation in these cases is abnormal methylation. Kane et al. (47) reported recently that methylation of the promoter region of hMLH1 correlated with the absence of hMLH1 expression in a small number of tumors and cell lines. Additional studies will be required to explore this phenomenon in more detail.

One of the questions remaining to be answered is: are there any real differences between MSS and MSI-L tumors? It may be that genomic instability is a general characteristic of colorectal cancer, and that MSS and MSI-L tumors represent opposite ends of the same tumor spectrum. Given the use of enough microsatellite markers, all such tumors may demonstrate low-frequency instability. On the other hand, there may be a measurable difference in the mutation rate between MSS and MSI-L tumors, with MSI-L tumors the result of mutator gene(s) other than hMSH2 or hMLH1. Although the involvement of defective mismatch repair in the MSI-L cases cannot be entirely ruled out, its role (if present), is likely to be very different compared with the MSI-H cases. Clearly, additional work will be required to more fully understand the differences, if any, between MSS and MSI-L tumors. Additionally, the cause of MSI in the MSI-L cases remains to be clarified.

MSI is not confined to tumors of the colon. Although the above criteria for defining MSI may apply to colon cancer, they are not necessarily applicable to other tumor systems. MSI has been reported in a variety of tumor types, particularly those within the HNPCC tumor spectrum. Although defective mismatch repair may be the cause of the instability observed in many of these sporadic tumors, this may not necessarily be the case for all tumors. As with colon cancer, some tumors with MSI may be composed of different subgroups, only some of which are due to defective mismatch repair. Others may result from entirely different mechanisms. For example, the MSI observed in sporadic endometrial cancer (48–51) suggests mechanisms other than defective mismatch repair. In this particular tumor system, the frequency of mutations within hMLH1 and hMSH2 has been reported to be low (49–51). In addition, Lim et al. (49) reported normal expression of hMLH1 and hMSH2 in sporadic endometrial cancers with MSI, suggesting that neither of these genes was responsible for the MSI phenotype.

In conclusion, our data indicate that there are at least two distinct mutator phenotypes in colorectal cancer: one that is characterized by a high rate of instability, MSI-H, and results primarily from an absence of hMLH1; and another that shows less frequent instability, MSI-L. The classification of tumors as either positive or negative for MSI can only consistently be made when certain criteria are applied, in particular the use of sufficient number of markers with inherently high levels of instability. Whether these differences are unique to colorectal cancer or will apply to other malignancies will be an important question to answer. These parameters for MSI classification should allow for a more uniform classification of MSI within varying tumor systems and will result in a better understanding of the role of defective DNA mismatch repair in the different human malignancies. Finally, determining why hMLH1 is preferentially involved in sporadic colorectal cancer may help us to elucidate mechanisms that result in the development of a subgroup of colorectal cancer.

ACKNOWLEDGMENTS

We thank Karen Erwin for excellent secretarial support.

REFERENCES


Downloaded from cancerres.aacrjournals.org on April 13, 2017. © 1998 American Association for Cancer Research.


Microsatellite Instability in Colorectal Cancer: Different Mutator Phenotypes and the Principal Involvement of \textit{hMLH1}

Stephen N. Thibodeau, Amy J. French, Julie M. Cunningham, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/58/8/1713

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.