Microsatellite Instability in Muir-Torre Syndrome

Ronald Honchel, Kevin C. Halling, Daniel J. Schaid, Mark Pittelkow, and Stephen N. Thibodeau

Departments of Laboratory Medicine and Pathology [R. H., K. C. H., S. N. T.], and Dermatology, and Division of Biostatistics [D. J. S.], Mayo Clinic Foundation, Rochester, Minnesota 55905

Abstract

Muir-Torre syndrome (MTS) is characterized by the presence of at least one sebaceous tumor and at least one visceral malignancy. Although a wide range of internal malignancies have been reported, the most frequently observed internal neoplasm is colorectal carcinoma. MTS and hereditary nonpolyposis colorectal carcinoma (HNPCC) share many clinical and pathological characteristics and thus may share similar genetic mechanisms of tumorigenesis. Recently, microsatellite instability (MIN) has been reported in tumor tissue from patients with HNPCC. In order to determine if tumors from MTS patients might also show MIN, we examined DNA extracted from paraffin-embedded tissues for the presence of MIN at (CA)n repeats on chromosomes 5q, 15q, 17q, and 18q. Data was obtained on 13 patients, 9 of which had at least one colorectal tumor. Of these, six demonstrated widespread MIN in all sebaceous and colorectal tumors examined, as well as in a transitional cell carcinoma of the renal pelvis, a prostatic adenocarcinoma and a keratoacanthoma. Overall, patients with MTS differed from patients without MIN in several respects, the most important of which include: (a) uniform presence and early onset of colorectal cancer (average age, 40 versus 70 years); (b) prolonged survival following diagnosis of visceral malignancy (median survival, 32 versus 11 years); and (c) a greater number of visceral and skin tumors. These data suggest that patients with MTS may be composed of at least two subgroups, each demonstrating different genetic, pathological and clinical features. Furthermore, the subgroup demonstrating MIN may share similar genetic mechanisms of tumorigenesis with patients having HNPCC, supporting the notion that these syndromes are allelic.

Introduction

MTS is defined by the development in an individual of at least one sebaceous gland tumor and a minimum of one internal malignancy (1, 2). Although a wide range of internal malignancies have been reported, the most frequently observed neoplasm is colorectal carcinoma (3, 4). Interestingly, MTS shares a number of clinical and pathological features with HNPCC syndromes Lynch I and Lynch II (5), including: (a) an autosomal dominantly inherited susceptibility to colorectal cancer; (b) onset of colorectal cancer at an early age; (c) tendency to develop right-sided colon cancers; and (d) prolonged survival after diagnosis of colorectal cancer. In addition, MTS shares with Lynch II a predisposition for the development of extracolonic tumors, especially those of the genitourinary tract. MTS differs from Lynch II syndrome, however, in the development of sebaceous tumors of the skin. As a result of the similarities noted above, Lynch et al. (6, 7) has suggested that the Muir-Torre syndrome may be a fuller phenotypic expression of the gene responsible for HNPCC.

MIN has recently been demonstrated in tumor DNA from patients diagnosed with HNPCC (8, 9) and from patients with sporadic colon cancer (10, 11). Although intriguing, the importance of MIN in tumorigenesis remains unknown. Because of the similar clinical and pathological presentations of MTS and HNPCC, we sought to determine if tumors from patients with MTS might also exhibit MIN. Our data suggests that the underlying genetic basis of MTS is likely to be heterogeneous in nature, with MIN being a prominent component in a subgroup of these patients. Additionally, the subgroup demonstrating MIN may represent an allelic variant of HNPCC.

Materials and Methods

Patient Population. Cutaneous biopsy specimens showing sebaceous adenoma, sebaceous epithelioma, or sebaceous carcinoma were identified from a review of all sebaceous neoplasms in the pathology archives of Mayo Clinic from 1923-1983 (3). Fifty-nine patients had at least one sebaceous tumor of the skin removed. Of these 59 patients, 25 also had at least one internal malignancy and thus were diagnosed as having MTS. Within this group, a total of 49 internal malignancies were identified, 25 of which were colonic, 9 urogenital, 5 hematologic, 4 breast, and 1 each for pancreas, jejunum, ovary, squamous cell carcinoma of inner ear, parotid (basal cell adenoma), and squamous cell carcinoma of the vocal cord. Additionally, colonic polyps were observed in 12, and a family history of carcinoma was detected in 18. Tumors were staged using the Astler-Coller modification of the Duke's staging system (12).

DNA Preparation. The acquisition of tissue and the review of patient histories were approved by the Mayo Institutional Review Board. We obtained as many blocks of paraffin-embedded tumors as possible from the MTS patients identified by Finan and Connolly (3). For each block, 5-μm thick sections were cut and placed on a glass microscope slide; standard hematoxylin and eosin staining was performed. Areas of the block selected for extraction (normal tissue or tumor tissue containing greater than 40% tumor cells) were carefully etched using a 19-gauge needle. Ten-μm thick sections were then cut until approximately 1 cm² of tissue was collected. The tissue sections were placed into a microfuge tube and deparaffinized by adding 1 ml of xylool for 10 min and pelleted by centrifugation at 8000 rpm for 10 min; the xylool was removed with a glass Pasteur pipette. The isolated tissue was washed twice with 1 ml of 95% ethanol and dried under vacuum. Two hundred μl of digestion buffer (10 mW Tris-HCl, pH 8, 50 mM KC1, 1.5 mM MgCl2, and 0.5% Tween 20) and 20 μl of glass beads (controlled pore glyceryl glass; Sigma, St. Louis, MO) suspended in H2O were added to each tube. The tubes were sonicated for 10 min at 45°C in a Branson 2210 sonicator. Twenty μl of 20 mg Proteinase K/ml H2O (Sigma) were added to each tube and incubated overnight at 55°C with shaking. Following this initial incubation, an additional 10 μl of Proteinase K was added to each tube, and the tubes were incubated for an additional 4 h at 55°C. Two phenol-chloroform and one chloroform extractions were performed on each tube, and an aliquot of the extracted aqueous layer was used as template for the PCR.

Analysis of Microsatellites by PCR. We examined the extracted DNA for genetic alterations at four separate microsatellites localized to chromosome arms 5q (APC), 15q (635/636), 17q (P53) and 18q (Mfd 26). The following primers were utilized for the PCR reaction: APC-F (ACTCATCCTGT-GATAAATCG) and APC-R (AGGACATAAGAGAGATTTAGTAGT), allele sizes 96-122 base pairs (13; 635/636-F (TTCAGCCTGAATGCACTGTGA) and 635/636-R (TTTACATCGGGACAGCT). The following primers were utilized for the PCR reaction: APC-F (ACTCATCCTGT-GATAAATCG) and APC-R (AGGACATAAGAGAGATTTAGTAGT), allele sizes 96-122 base pairs (13; 635/636-F (TTCAGCCTGAATGCACTGTGA) and 635/636-R (TTTACATCGGGACAGCT). The following primers were utilized for the PCR reaction: APC-F (ACTCATCCTGT-GATAAATCG) and APC-R (AGGACATAAGAGAGATTTAGTAGT), allele sizes 96-122 base pairs (13; 635/636-F (TTCAGCCTGAATGCACTGTGA) and 635/636-R (TTTACATCGGGACAGCT). The following primers were utilized for the PCR reaction: APC-F (ACTCATCCTGT-GATAAATCG) and APC-R (AGGACATAAGAGAGATTTAGTAGT), allele sizes 96-122 base pairs (13; 635/636-F (TTCAGCCTGAATGCACTGTGA) and 635/636-R (TTTACATCGGGACAGCT). The following primers were utilized for the PCR reaction: APC-F (ACTCATCCTGT-GATAAATCG) and APC-R (AGGACATAAGAGAGATTTAGTAGT), allele sizes 96-122 base pairs (13; 635/636-F (TTCAGCCTGAATGCACTGTGA) and 635/636-R (TTTACATCGGGACAGCT).
Statistical Methods. Age of onset was compared by the exact Wilcoxon rank sum statistic (15). Survival distributions were estimated by the Kaplan and Meier method (16), and survival comparison was made by the log rank statistic (17).

Results and Discussion

As many paraffin blocks as possible were obtained from the MTS patients identified by Finan and Connolly (3). Twelve of the 25 patients could not be assessed for MIN for one of the following reasons: (a) unavailability of paraffin-embedded tissue; (b) inability to amplify the extracted DNA by PCR; or (c) insufficient tumor cell content (less than 40% of the cells) in the paraffin-embedded material. Some patients developed additional tumors following the analyses by Finan and Connolly (3), and these were included in our study.

We were able to assess 13 of the 25 MTS patients for MIN in at least 1 sebaceous tumor or one visceral malignancy. The clinical and pathological findings in these 13 patients are summarized in Table 1 (note that the original patient case numbers from the Finan and Connolly study have been retained). Those tumors with PCR data are indicated with a superscript c, and a summary of these analyses are shown in Table 2. Six patients had tumors which exhibited MIN in at least three of the four loci tested (Group I). Examples of MIN in a

<table>
<thead>
<tr>
<th>Case #</th>
<th>No. of sebaceous tumor(s)</th>
<th>Other skin tumors (n)</th>
<th>Site</th>
<th>Histology</th>
<th>Stage</th>
<th>Age</th>
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<tr>
<td>4</td>
<td>5</td>
<td>BCC</td>
<td>Cecum</td>
<td>ACA</td>
<td>B2</td>
<td>53</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>AK (&gt;50)</td>
<td>Ascending colon</td>
<td>? (Outside institution)</td>
<td></td>
<td>42</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>BCC</td>
<td>Transverse colon</td>
<td>ACA</td>
<td>B2</td>
<td>38</td>
</tr>
<tr>
<td>23</td>
<td>6</td>
<td>AK</td>
<td>Rectum</td>
<td>ACA</td>
<td>B1</td>
<td>42</td>
</tr>
<tr>
<td>24</td>
<td>5</td>
<td>AK</td>
<td>Cecum</td>
<td>ACA</td>
<td>C2</td>
<td>23</td>
</tr>
<tr>
<td>25</td>
<td>1</td>
<td>AK</td>
<td>Descending colon</td>
<td>ACA</td>
<td>C2</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>BCC (2)</td>
<td>Bladder</td>
<td>TCC</td>
<td>C2</td>
<td>79</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>Colon (splenic flexure)</td>
<td>ACA</td>
<td>C2</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>Cecum (hepatic flexure)</td>
<td>ACA</td>
<td>C2</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>1</td>
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<td>B2</td>
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<tr>
<td>19</td>
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<td>Breast</td>
<td>ACA</td>
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<td>1</td>
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<td>ACA</td>
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<tr>
<td>22</td>
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<td>Breast</td>
<td>ACA</td>
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</table>

| a | Stage of colorectal cancer. |
| b | Age at diagnosis of visceral malignancy. |
| c | PCR data obtained. |
| d | ACA, adenocarcinoma; AK, actinic keratosis; BCC, basal cell carcinoma; CLL, chronic lymphocytic leukemia; EC, epidermoid cyst; IFK, inverted follicular keratosis; KA, keratoacanthoma; NHL, non-Hodgkin’s lymphoma; RCC, renal cell carcinoma; SCC, squamous cell carcinoma; SK, seborrhoeic keratosis; TCC, transitional cell carcinoma; VK, verrucal keratosis. |
Variety of tumors are shown in Fig. 1. One patient (case 21) exhibited MIN in any of the tumors examined. The remaining six patients failed to demonstrate MIN in any of the tumors examined (Group II). The age of onset for the first visceral malignancy, survival after the onset of this malignancy, and presence or absence of family history of cancer for the 13 patients are summarized in Table 3.

Patients with tumors that demonstrated MIN had an average of 4.8 sebaceous tumors, 19 skin tumors of other types, and 3.5 visceral malignancies, whereas patients with tumors lacking MIN had an average of 1.0 sebaceous tumors, 1.3 skin tumors of other types, and 1.3 visceral malignancies. The average age of diagnosis for the first sebaceous tumor was 62 years (range, 55–73 years) in Group I and 70 years (range, 57–85 years) in Group II. In all Group I patients, the first sebaceous tumor occurred after the development of the first visceral malignancy (average of 22 years with a range of 2–37 years). On the other hand, the average age at which the first sebaceous tumor was diagnosed in a Group II patient was the same as the age of diagnosis for the first visceral malignancy (range, 11 years before to 11 years after). No difference was observed in the number of colonic polyps detected in patients with or without MIN.

Overall, our findings suggest that the underlying molecular basis of MTS is likely to be heterogeneous in nature, with the presence of at least two subgroups of patients, those with (Group I) and those without (Group II) widespread MIN in their tumors. A comparison of the clinical and pathological features of these two groups of patients reveals several interesting differences. First, Group I patients had an earlier age of onset for their first visceral malignancy (average age, 40 years; range, 23–53 years) compared to Group II patients (average age, 70 years; range, 62–79 years), a difference that was highly significant ($P = 0.001$). Second, survival time, with death from any cause, was significantly shorter ($P = 0.04$) for patients without MIN (median survival, 11 years) compared to patients with MIN (median survival, 32 years). Although statistically significant, these results should be interpreted with caution because of the small patient population: four deaths per six patients in Group I versus five deaths per seven patients in Group II. One patient in each group died without evidence of cancer (one with Alzheimer’s disease and one from heart disease). If these patients are censored at the time of their death, the statistical comparison was no longer significant ($P = 0.09$). Third, all Group I patients had at least one colorectal cancer as one of their visceral malignancies, while this was not necessarily the case for Group II patients. Among the four patients in Group II not having a colon cancer, two had breast cancer, one had a small lymphocytic lymphoma, and another a bladder cancer. Fourth, all Group I patients had a family history of cancer, while this was true for only four of the seven patients in Group II; and, finally the development of numerous additional skin and visceral malignancies was common in Group I while uncommon in Group II.

As initially noted by Lynch et al. (6, 7), patients with MTS and Lynch syndrome share many common clinicopathological features. In this study, we demonstrate that genomic instability as assessed by
MIN is an additional common feature between these two disorders. These data, therefore, further support the hypothesis that these syndromes may be allelic in nature (6, 7) but possibly only for a subset of MTS patients (i.e., those demonstrating MIN). The gene responsible for some families with HNPCC (allelic heterogeneity has been observed) has recently been localized to chromosome 2 by genetic linkage analysis (18). Given the presence of MIN in a subset of MTS, it will be important to explore the role of this gene in this disorder either by linkage analysis or, once identified, by direct analysis of the gene.

While we were unable to obtain PCR data on at least one sebaceous tumor for all patients in Group II, the lack of the typical clinicopathological features along with the lack of MIN in the tumors examined argues against their being classified as Lynch II variants. Thus, as originally defined, the Muir-Torre syndrome appears to include at least two subgroups of patients: one subgroup exhibiting the clinical features of Lynch II syndrome and demonstrating MIN; and the other not exhibiting the features of Lynch II syndrome and not demonstrating MIN. This apparent heterogeneity of MTS may explain the intermediate age of onset of colorectal cancer that has been reported in MTS patients (3, 4). Alternatively, some patients in Group II may be incorrectly classified as having MTS. For example, two patients in this study (cases 19 and 22) were categorized as having MTS due to the development of a Meibomian gland adenoma and a Meibomian gland carcinoma, respectively. Meibomian gland tumors are sebaceous tumors of the eyelid and are thought by some investigators to fulfill the diagnostic criteria for MTS (19, 20). Although it is difficult to draw firm conclusions from just two tumors, the absence of MIN in the Meibomian gland adenoma and Meibomian gland carcinoma would argue that the presence of such lesions as the sole sebaceous neoplasm may not be sufficient to make the diagnosis of MTS.

The presence of MIN in a group of tumors that are seemingly unrelated (e.g., colorectal carcinoma, transitional cell carcinoma, and sebaceous adenoma) and the lack of MIN in some of the tumors in Group I is interesting and unexplained. Perhaps the tissues from which these MIN+ neoplasias arise are more susceptible to a "mutator gene effect" because of the influence of other environmental factors, such as UV exposure or various carcinogens. Alternatively, other tissue-specific modifier genes may play a role in neoplasia of these tissues. In any case, it appears that each of the tumors demonstrating MIN in MTS shares a common underlying molecular basis of carcinogenesis.

The indolent nature of the tumors in MTS and HNPCC is one of the most intriguing features of these syndromes. In the present study, prolonged survival was observed within Group I (MIN+) despite the fact that the majority of these patients developed metachronous colon cancers, some of advanced stage. For instance, case 24 was a female who developed a cecal carcinoma (modified Dukes B1) at age 23, a metastatic renal cell carcinoma in case 6 and a chronic lymphocytic leukemia in case 9) that did not demonstrate MIN. Unlike tumors demonstrating microsatellite instability, these cancers did not behave indolently and eventually led to the patients’ deaths. The absence of MIN in these malignancies argues that some of the tumors that occur in MTS patients could arise through separate genetic mechanisms. Thus, while patients with MTS are predisposed to the development of malignant tumors which behave indolently, they do not appear to be "resistant" to the development of cancers that appear to arise through different genetic mechanisms and which might otherwise behave more aggressively.

In summary, we show that a subset of MTS patients develop tumors with widespread MIN. In general, these patients exhibit the clinical and pathological features of Lynch syndrome II and thus may represent a phenotypic variant of the Lynch II syndrome. Those patients with MTS not demonstrating MIN have quite different clinicopathological features, suggesting a different genetic basis for their disease.
References


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