The Mechanism of Microsatellite Instability Is Different in Synchronous and Metachronous Colorectal Cancer

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MLH1 promoter hypermethylation has been described as the primary mechanism for high-frequency microsatellite instability (MSI-H) in sporadic colorectal cancers (CRCs). The underlying molecular mechanism for microsatellite instability (MSI) in synchronous and metachronous CRCs is not well described. A total of 33 metachronous CRC patients and 77 synchronous CRC patients were identified from 2884 consecutive patients undergoing cancer surgery in an academic center. Evaluable tumors were tested for MSI, immunohistochemistry for MLH1 and MSH2 protein expression, and hypermethylation of the MLH1 promoter. MSI-H tumors were found in 12 (36%) metachronous CRC patients and 29 (38%) synchronous CRC patients. MSI-H metachronous CRC patients were younger at index cancer diagnosis (64 vs. 76 years, $P = 0.01$) and more often were diagnosed before 50 years of age (4 of 12 vs. 0 of 29, $P = 0.005$). Loss of MLH1 expression associated with promoter hypermethylation was common in all patients, although more common in MSI-H synchronous patients (50% metachronous vs. 83% synchronous, $P = 0.03$). Overall, MLH1 promoter hypermethylation was seen in 7 of 17 (41%) metachronous and 44 of 54 (81%) synchronous MSI-H CRCs tested ($P = 0.004$). Although MSI occurred with equal frequency among patients with synchronous and metachronous CRCs, the underlying mechanism for MSI was different. Observed differences in MLH1 promoter hypermethylation and patient characteristics suggest most MSI-H synchronous CRCs in our population were sporadic in origin. In contrast, more MSI-H metachronous CRCs were associated with patient and tumor characteristics suggestive of underlying hereditary nonpolyposis CRC. (J GASTROINTEST SURG 2005;9:329–335)

KEY WORDS: Colorectal cancer, synchronous colorectal cancer, metachronous colorectal cancer, multiple tumors, hereditary nonpolyposis colorectal cancer, microsatellite instability, MLH1, MSH2, promoter methylation, epigenetic modification

Individuals with an inherited predisposition to cancer development are at an increased risk of developing multiple colorectal cancers (CRCs). Hereditary nonpolyposis colorectal cancer (HNPCC) is the most common hereditary colon cancer syndrome.[1] It is due to an autosomal dominant germline mutation in one of the DNA mismatch repair genes and accounts for approximately 3–5% of CRCs.[2–4] In over 90% of cases, the mutation is in the mismatch repair genes *MLH1* or *MSH2*.[5] Characteristic features include early-onset CRCs, predominance of cancers located proximal to the splenic flexure, and extracolonic malignancies.[6] Multiple colonic tumors are another common feature and occur in 20–40% of affected individuals. Multiple colonic tumors, however, can also occur in the absence of HNPCC. Up to 5% of patients with sporadic CRC have multiple CRCs (synchronous or metachronous).[6] Accordingly, the Bethesda Guidelines recommend testing of tumors from synchronous and metachronous CRC patients

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for the presence of microsatellite instability (MSI). Individuals whose tumors demonstrate MSI are at risk for having HNPCC and are referred for more specific and expensive germline testing to confirm or reject the diagnosis.[3,4]

Not all MSI cancers are due to HNPCC. Epigenetic inactivation of the *MLH1* gene by hypermethylation of its promoter has been recognized as the principal mechanism of gene inactivation in high-frequency microsatellite instability (MSI-H) sporadic CRC. This mechanism explains in part the discrepancy between the 10–20% rate of MSI seen in unselected patients with CRC and the 2% rate of HNPCC among these same patients.[7,8] The role that MLH1 promoter hypermethylation may play in the MSI-H phenotype in synchronous and metachronous cancer is unknown.

Little distinction has been made between these two types of multiple cancers with respect to the risk of underlying hereditary cancer. The aim of this study was to analyze a large series of unselected metachronous CRCs and assess the frequency of MSI, describe the molecular mechanism responsible for the MSI phenotype, and assess the contribution of MLH1 promoter hypermethylation to the MSI-H phenotype. We compared these results with a previously described group of synchronous CRC.[9]

PATIENTS AND METHODS

Study Population and Data Collection

Patients were identified from a series of 2884 consecutive patients with CRC treated at the University of Minnesota from January 1987 to December 1998. *Metachronous CRCs* were defined as occurring more than 12 months apart. *Synchronous CRCs* were defined as simultaneously diagnosed multiple invasive adenocarcinomas located in different anatomic segments of the large bowel. Patients with carcinoma in situ, inflammatory bowel disease, local recurrences, and familial adenomatous polyposis were excluded. Tumors were classified as proximal and distal, based on their location in relation to the splenic flexure. A total of 46 patients (1.6%) with metachronous CRC and 85 patients (2.9%) with synchronous CRC were identified.

Paraffin blocks from the surgical specimens were available in 33 of 46 patients with metachronous CRC. In 10 patients, paraffin blocks from the index and second tumors were available (21 tumors). In the remaining 23 patients, only paraffin blocks for the second tumors were available (24 tumors). In the synchronous group, paraffin blocks were available in 77 of 85 patients (170 tumors). Patients with available tumors constitute the study population.

Family history of CRC was obtained from patient self-report noted in the University of Minnesota Colorectal Cancer Database and by reviewing all patient medical records. We attempted to confirm family history data with a mailed questionnaire and telephone interview with the patient or next of kin. The questionnaire specifically inquired about the total number of first-degree relatives and the number of relatives with HNPCC-related disease. Completed family history information was available for 25 of 33 patients (76%) with metachronous CRC and 59 of 77 (77%) patients with synchronous CRC. Among patients with at least one MSI-H tumor, completed family history was available in 11 of 12 patients (92%) with metachronous CRC and 22 of 29 patients (76%) with synchronous CRC.

Tumor Analysis

Methods of DNA extraction, MSI analysis, immunohistochemistry, and MLH1 promoter methylation have been described previously.[13] Briefly, representative samples of hematoxylin and eosin–stained normal mucosa and cancer tissue were microdissected into separate Eppendorf tubes (Eppendorf, Inc, Hamburg, Germany) using a sterile scalpel. Individual DNA samples from tumors were amplified by polymerase chain reaction (PCR) using a set of five fluorescent-labeled microsatellite markers (*BAT25*, *BAT26*, *D2S123*, *D5S346*, and *D17S250*) recommended by the National Cancer Institute Workshop on Microsatellite Instability in Colorectal Cancer and analyzed using an automated sequencer.[14] PCR products were separated in 6% denaturing polyacrylamide gels and visualized with silver staining. Matched tumor and normal DNA were compared. The presence of new microsatellite alleles at two or more loci was scored as MSI-H. Tumors were otherwise considered microsatellite stable (MSS).[10]

Formalin-fixed, paraffin-embedded 5μm-thick sections containing both malignant tissue and normal mucosa were stained for presence (positive) or absence (negative) of MLH1 and MSH2 expression using monoclonal antibodies against MLH1 (clone G168-15; PharMingen, San Diego, CA) or MSH2 (clone EF11; Oncogene Sciences, Cambridge, MA), respectively.

MLH1 promoter methylation status was determined by a methylation-specific PCR assay described by Herman et al.[11] Approximately 100 ng of genomic DNA was extracted from each tumor. DNA was denatured and modified samples were purified using the Wizard DNA purification kit (Promega, Madison, WI). Separate PCRs were subsequently performed with MLH1 promoter methylated or unmethylated-