Low Frequency of Lynch Syndrome Among Young Patients With Non-Familial Colorectal Cancer

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BACKGROUND & AIMS: Colorectal cancer (CRC) is uncommon in individuals <50 years old. Lynch syndrome is caused by germline mutations in DNA mismatch repair (MMR) genes and associated with early-onset CRC, but little is known about the proportion of young patients with apparently sporadic CRC who actually have Lynch syndrome. We examined patterns of microsatellite instability (MSI) and MMR genes among patients <50 years old with non-familial CRC (patients with not more than 1 family member with CRC). **METHODS:** Tissue specimens were collected from 75 CRC patients <50 years old (mean age, 34.5 years) and analyzed using immunohistochemical analyses of MLH1, MSH2, MSH6, and PMS2. MSI and mutations in *BRAF* and *KRAS* were also analyzed. **RESULTS:** Most cancers (72%) arose in the distal colon. MSI was detected in 21% of the samples, and loss of 1 or more MMR proteins was observed in 21%. Interestingly, only 38% of the MMR-deficient CRCs lost either MLH1 or MSH2, whereas 63% of the MMR-deficient CRC samples lost either PMS2 or MSH6. All 11 CRC samples that had lost MSH2, MLH1, or PMS2 had MSI, but only 2 of the 5 tumors that lost only MSH6 had MSI. There were no *BRAF* mutations in any tumor. **CONCLUSIONS: In young patients with apparently sporadic CRC, most tumors arise in the distal colon; only 21% have features of Lynch syndrome. Loss of MSH6 or PMS2 occurred in 13.3% of these tumors. Most tumors that lose MSH6 will not be detected in screens for MSI; CRC screening might be modified to identify more patients with Lynch syndrome.**

Keywords: Colon Cancer; Young Patients; Familial Colorectal Cancer.

Colorectal cancer (CRC) is a common disease with 5.29% lifetime risk in the United States. The disease is typically associated with aging. The median age for CRC from 2001–2005 according to the United States Surveillance Epidemiology and End Results database was 71 years; 4.8% of CRCs occurred by age 45 and 16.9% by age 55.[1] The disease is uncommon in young people.

A number of specific diseases are associated with CRCs that occur before age 50. These include inflammatory bowel disease, familial adenomatous polyposis (FAP), and Lynch syndrome. However, the relative contribution of each of these has not been systemically explored, although there are important implications associated with each of these. Ulcerative colitis and most cases of FAP are readily identified by their phenotypic features, and both require total proctocolectomies. However, Lynch syndrome does not have a characteristic premorbid phenotype, and inadequate management typically occurs when the diagnosis is not known before treating the tumor.

Lynch syndrome is a familial predisposition to cancers of the colon, rectum, uterus, ovary, stomach, and other organs.[2] The CRCs have some phenotypically distinctive features. They occur at an average age between 40 and 50 years, and approximately two-thirds of these tumors occur proximal to the splenic flexure.[3] The diagnosis of Lynch syndrome is suspected when there is a family history that involves 3 or more individuals with CRC and at least 1 patient with CRC is <50 years old,[4] but the diagnosis is definitively established by finding a deleterious germline mutation in a DNA mismatch repair (MMR) gene.[5] Germline mutations in any of 4 DNA MMR genes (*MSH2*, *MLH1*, *MSH6,* and *PMS2*) cause Lynch syndrome, and this diagnosis has been definitely made in 2.2% of a population-based survey of CRC patients by using existing sequencing analysis.[6] However, as the analytical approaches to these genes become more sensitive, the proportion of all CRC patients who have Lynch syndrome might increase to perhaps 3%–4%.

If a patient is known to have Lynch syndrome and develops CRC, the recommended strategy is to perform a subtotal colectomy, because the risk of metachronous tumors is so high.[7] One study reported a 40% risk of a second primary CRC within 7 years of the first tumor.[8] Also, women with Lynch syndrome need to understand that their risk of endometrial cancer might be even higher than their risk of CRC,[9] underscoring the implications of making a correct and timely diagnosis. Moreover, there are important implications for the first-degree relatives of patients with Lynch syndrome.

However, this diagnosis is a particular challenge when there is no family history to rely on (small families, inadequate

Abbreviations used in this paper: CRC, colorectal cancer; FAP, familial adenomatous polyposis; IHC, immunohistochemistry; MMR, mismatch repair; MSI, microsatellite instability; PCR, polymerase chain reaction; ROC, receiver operating curve.

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information), or if there has never been a germline mutation documented in the family. One should have an increased suspicion for this disease when CRC occurs in a young individual. However, the likelihood of this diagnosis and the optimal approach to screening for the disease have never been established. This study examined a cohort of CRC patients ≤50 years old, from which we excluded those with a family history suggestive of Lynch syndrome and those with FAP or inflammatory bowel disease, and screened the tumor tissue for the characteristic features of defective DNA MMR. Interestingly, we found that only a relatively small percentage of these patients had Lynch syndrome, and that the distribution of genes involved was unexpected. Furthermore, those young patients with CRC who did not have Lynch syndrome had a marked clustering of tumors in the distal colon. We found other unique features by looking for mutations in *BRAF* and *KRAS* genes. These findings have important implications for the care of young patients with CRC.

Materials and Methods

Tissue Specimens

This study began with a cohort of 85 patients ≤50 years old with primary CRC in which the surgical treatment occurred at the Baylor University Medical Center, Dallas between 1986 and 2004. Patients with diagnoses of inflammatory bowel disease or FAP and patients who had more than 1 family member known to have CRC were excluded from analysis. None of the patients with other Lynch syndrome–associated cancers was excluded from data analysis. Patients signed a protocol-specific informed consent for use of their tissues, and Institutional Review Board approval was granted for the studies, which were performed on anonymized samples.

Microdissection and DNA Amplification

Serial sections from formalin-fixed paraffin-embedded neoplastic and matched normal tissues (5 μm) were stained for pathologic analysis, and representative normal and tumor regions were identified. Normal (non-tumor) control tissue was obtained from histologically normal mucosa or lymph nodes. Genomic DNA was isolated from the tissue microdomains after serial rehydration of the slides. The hydrated tissues were digested with proteinase K and followed by DNA extraction using the QIAamp DNA mini-kit (Qiagen, Valencia, CA), according to the manufacturer's instructions.

Microsatellite Instability Analysis

Microsatellite analysis of the extracted DNA specimens was performed by polymerase chain reaction (PCR) amplification using a panel of 5 mononucleotide repeat markers in a pentaplex PCR.[10] The 5 markers in the pentaplex PCR system were mononucleotide repeats: BAT25, BAT26, NR21, NR24, and NR27, as previously described,[10,11] and allelic sizes were estimated by using Genescan 2.1 software (Applied Biosystems, Foster City, CA). Microsatellite instability (MSI) was defined when 2 or more of the 5 markers showed allelic shifts; those with 1 were called microsatellite low (MSI-L) and those with 0 mutated alleles were microsatellite stable (MSS).

Mismatch Repair Protein Immunohistochemistry

We examined the protein expression of MLH1, MSH2, PMS2, and MSH6 in all 75 tumor tissues by immunohistochemistry (IHC) staining with the DAKO EnVision System-HRP polymer system kit (Dako Cytomation Inc, Carpinteria, CA). One block of formalin-fixed, paraffin-embedded tumor tissue was selected per case. Before IHC staining, antigen retrieval was performed by immersing sections in 10 mmol/L concentration of citrate buffer, pH 6.0, and boiling in a pressure cooker for 5 minutes. Sections were thereafter incubated for 1 hour with appropriate dilutions of mouse monoclonal antibodies against MLH1 (clone 13271A; BD Pharmingen, San Diego, CA), MSH2 (clone FE11; Oncogene Research Products, Boston, MA), PMS2 (clone A37; BD Pharmingen), and MSH6 protein (clone 44; BD Transduction Laboratories, Lexington, KY). The peroxidase reaction was developed by using diaminobenzidine tetrachloride as the chromogen. Tumor cells were judged to be negative for protein expression only if they lacked staining in a sample in which non-neoplastic colonocytes and stroma cells were positively stained. Tumors lacking MLH1 or MSH2 expression (ie, the "major" DNA MMR protein products) characteristically lack PMS2 or MSH6 expression, respectively, (ie, the "minor" DNA MMR protein products) because of heterodimeric protein stabilization.[12,13] Hence, we classified tumors lacking both MLH1 and PMS2 expression as MLH1-deficient and tumors lacking both MSH2 and MSH6 expression as MSH2-deficient. This was distinguished from those tumors with isolated absence of expression of PMS2 or MSH6, which suggests specific abnormalities in the *PMS2* and *MSH6* genes.

Analysis for Mutations in BRAF *and* KRAS

Mutational analysis of *BRAF* and *KRAS* was performed by PCR and sequencing as previously reported.[14] Analysis was specifically focused on the V600E mutation of *BRAF* and codons 12 and 13 of *KRAS*.

Statistical Analysis

We used logistic regression analysis to examine the performance of the different strategies to define MSI status in the MMR-deficient tumors. To examine the relationship between the markers, multivariate correlation and hierarchical clustering analysis were performed using standardized absolute difference length between tumor allele and germline allele, as described previously.[11] Analyses were performed with the use of JMP software, version 6.0 (SAS Institute, Cary, NC). All reported *P* values are two-sided, and a *P* value of less than .05 was considered statistically significant.

Results

Patient Characteristics

A total of 75 patients had CRC; were ≤50 years of age; did not have inflammatory bowel disease, FAP, or family history that suggested Lynch syndrome; and the colonic resection specimens were available for analysis. The mean age of the patients was 34.5 years (95% confidence interval, 33.2–35.8). Forty-one of the patients were women (55%), and 34 were men (45%). Twenty-one of the CRCs (28%) were located proximal to the splenic flexure, and 54 (72%) were distal. Forty-four (58.6%) were lo-