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Microsatellite Instability in Nonneoplastic Mucosa from Patients with Chronic Ulcerative Colitis[1]

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Abstract

Microsatellite instability (MIN) has been detected in many cancer types; however, recently we also observed it in the nonneoplastic but inflammatory setting of pancreatitis. Consequently, we sought to examine whether MIN was present in another inflammatory condition, ulcerative colitis (UC). MIN was found in 50% of UC patients whose colonic mucosa was negative for dysplasia, 46% of those with high-grade dysplasia, and 40% of those with cancer but in none of the nine ischemic or infectious colitis controls ($P < 0.03$). Thus, UC patients may have MIN within mucosa that has no histological evidence of neoplastic change. MIN in this setting may reflect the inability of DNA repair mechanisms to compensate for the stress of chronic inflammation, and may be one mechanism for the heightened neoplastic risk in UC.

Introduction

Microsatellites are simple repetitive sequences of DNA that are scattered throughout the genome. These sequences are stably inherited, vary from individual to individual, and have a relatively low mutation rate. Instability within these sequences is a marker of genome-wide mutations and DNA repair deficiencies (1). MIN[3] has been detected in cancers associated with the HNPCC syndrome, as well as in a variety of sporadic cancers, including gastric, endometrial, and colorectal cancers associated with UC (2–5). However, MIN may not simply be limited to malignant and premalignant tissue. We recently detected MIN in the nonneoplastic setting of pancreatitis. Its prevalence in pancreatitis (100%) was greater than in pancreatic adenocarcinoma (40%) or in most other cancers (6). To establish whether MIN was present in another disease in which inflammation may precede development of carcinoma, we studied MIN in the nonneoplastic and neoplastic mucosa of UC.

Materials and Methods

Patients. Colonic biopsy specimens from 28 patients with LT-UC (duration of 8 years or more), and 6 patients with ST-UC (duration of 7 years or less), were evaluated for MIN. All of the LT-UC patients were considered to be at high risk for cancer because a heightened neoplastic risk is well established in patients with extensive disease of long duration. Extent of disease was determined histologically; most of the patients had pancolitis (Table 1). The 28 LT-UC patients were categorized according to the highest degree of histological abnormality found at colectomy or colonoscopy, and included 10 who were negative for dysplasia or cancer, 13 with HGD, and 5 with cancer. The duration of disease in the LT-UC patients ranged from 8 to 52 years with an average duration of 20 years. Three patients (UC-17, UC-24, and UC-25) had an onset of symptoms that was vague, but the disease had been present for many years. To avoid an error of omission, these patients were included in the LT-UC group; however, they were not included in the calculation of mean years of disease duration. The 6 ST-UC patients were evaluated by colonoscopy and biopsy, and all had mucosal biopsy specimens that were negative for dysplasia or cancer. Duration of ST-UC disease ranged from 1 to 3 years, with an average duration of 2.2 years.

Among both the LT-UC and ST-UC patients, a sufficient number of biopsy specimens were obtained at each colonoscopic examination to ensure that dysplasia or cancer would not be missed if it were present (an average of 48 biopsies obtained per colonoscopy and 120 biopsies obtained per colectomy; Ref. 7). Samples to be evaluated for MIN were selected from the sites of highest histological abnormality (HGD or cancer). In addition, several of the patients with cancer/HGD also had randomly selected additional sites that were histologically negative for dysplasia tested for MIN. In patients who did not have dysplasia or cancer, random sites were selected for study.

The nine negative controls included colonic biopsy specimens obtained from seven patients with ischemic colitis and 2 patients with infectious colitis.

MIN. Five-μm paraffin sections of colonic mucosa on glass slides were microdissected to enhance the yield of neoplastic cells or, in the case of histologically negative samples, to recover the epithelial cells. Matching constitutional tissue was obtained from lymph nodes or smooth muscle from the bowel wall or peripheral blood lymphocytes. DNA was extracted and amplified by PCR using primers directed at the following microsatellite loci: *D2S119*, *D2S123*, *D2S136*, *D3S1067*, *D5S346*, *D6S87*, *D8S255*, *D13S175*, *D17S87*, *D17S261*, *p53* (intron 1), *D18S34*, and *D18S35* (8, 9). Eight to 12 loci were tested, and an average of 7 loci was evaluable per specimen. Reactions were performed under standard conditions, and the PCR products were separated using a 4% polyacrylamide gel. Band shifts between the sample specimen and the matching constitutional specimen were evaluated blindly without knowledge of the diagnosis of the patient; ambiguous samples were either repeated or not included in the analysis. A band shift was defined as the presence of bands in the PCR product of sample tissue that were not visible in corresponding constitutional DNA. MIN was defined as the presence of one or more band shift, in addition to the presence of the normal allele, in the target tissue. All autoradiographs were blindly reevaluated for MIN by a second laboratory.

Statistical Methods. Fisher's exact test was performed using statistical software (Graphpad Instat, Graphpad Software, San Diego, CA). Probability values were two-tailed, with a P value of ≤ 0.05 regarded as statistically significant.

Results

MIN at One or More Loci. In the LT-UC group, MIN was detected in 6 of 10 (60%) patients whose colonic mucosa was histologically negative, in 11 of 13 (85%) patients with HGD, and in 2 of 5 (40%) patients with cancer (Fig. 1 and Table 1). In the ST-UC group, MIN was detected in 5 of 6 (83%) patients, all of whom had mucosal biopsy specimens that were histologically negative for dys-

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[3] The abbreviations used are: MIN, microsatellite instability; UC, ulcerative colitis; LT-UC, long-term ulcerative colitis; ST-UC, short-term ulcerative colitis; HGD, high-grade dysplasia; HNPCC, hereditary nonpolyposis colon cancer.

Table 1 *MIN in LT-UC*

Patient	Duration (years)	Ext[a]	MIN	Sites + for MIN
UC-Cancer				
LT-UC 13	13	Pan	5 of 10	*D2S123, D2S136, D2S119, D13S175, D18S34*
LT-UC 14	20	Pan	0 of 9	None
LT-UC 23	24	Pan	0 of 7	None
LT-UC 24	NA	NA	0 of 6	None
LT-UC 28	52	Pan	3 of 10	*DS2136, D17S261, D6S87*
UC-HGD				
LT-UC 1	22	L	0 of 8	None
LT-UC 2	11	Pan	1 of 8	*p53* (intron 1)
LT-UC 3	8	L	6 of 8	*D5S346, D3S1067, D17S87, D13S175, D17S261, D18S34*
LT-UC 4	20	Pan	2 of 8	*D5S107, D5S346*
LT-UC 5	19	Pan	1 of 8	*D2S136*
LT-UC 6	20	Pan	6 of 8	*D2S119, D2S136, D5S346, D18S34, D18S35, p53* (intron 1)
LT-UC 8	16	Pan	3 of 8	*D2S119, D2S136, D18S35*
LT-UC 9	18	Pan	1 of 8	*D5S107*
LT-UC 15	37	Pan	2 of 8	*D2S136, D18S34*
LT-UC 16	20	Pan	3 of 8	*D2S136, D3S1067, D5S346*
LT-UC 17	NA	Pan	1 of 5	*D13S175*
LT-UC 25	NA	NA	1 of 9	*D13S175*
LT-UC 27	16	Pan	0 of 3	None
UC-Negative				
LT-UC 7	21	Pan	4 of 11	*D2S123, D2S136, D2S119, D17S261*
LT-UC 10	22	Pan	1 of 9	*D17S261*
LT-UC 11	20	Pan	0 of 9	None
LT-UC 18	8	L	0 of 6	None
LT-UC 19	20	Pan	0 of 6	None
LT-UC 21	9	L	0 of 3	None
LT-UC 29	25	Pan	3 of 7	*D2S136, D3S1067, D18S34*
LT-UC 30	10	Pan	3 of 7	*D2S136, D6S87, D13S175*
LT-UC 31	29	Pan	3 of 6	*D2S123, D6S87, D13S175*
LT-UC 32	22	Pan	2 of 8	*D3S1067, D6S87*

[a] Extent of disease.
[b] Pan, pancolitis; L, left-sided disease; NA, information not available.

plasia (Table 2). MIN was not detected at any locus of any sample from the ischemic or infectious colitis patients (Table 3).

MIN at Two or More Loci. In patients with LT-UC, MIN was detected at two or more loci in 5 of 10 patients (50%) whose mucosa was histologically negative for dysplasia. Family histories were available for two of these LT-UC patients, and they were negative for colon cancer (no colon cancer and no HNPCC). MIN was detected at two or more loci in 6 of 13 LT-UC patients (46%) who had HGD and in 2 of 5 (40%) who had cancer. (Tables 1 and 4; differences not significant). In the six patients with ST-UC, MIN was detected at two or more loci in two of the six (33%; Tables 2 and 4). Family histories were available for both ST-UC patients who demonstrated MIN, and neither of them had first-degree relatives with colon cancer (no colon cancer and no HNPCC). MIN was not detected in any sample of ischemic or infectious colitis, as noted above (Table 3), whereas 50% of nonneoplastic LT-UC samples tested had MIN at two or more loci ($P \leq 0.03$; Table 4).

MIN in Negative Sites from Patients with Cancer or HGD. Four of the LT-UC patients with cancer or HGD and MIN were randomly selected for further study (LT-UC 4, LT-UC 13, LT-UC 16, and LT-UC 28). Two histologically negative sites, remote from the areas of HGD/cancer, were randomly selected from each of these patients and tested for the presence of MIN. Two of the patients had evidence of MIN at two or more loci in all sites tested (LT-UC 4 and LT-UC 28). One patient had evidence of MIN at a HGD site but not at the two randomly selected nonneoplastic sites (LT-UC 16), and the last patient had MIN at two or more loci in a cancer site and in one nonneoplastic site, whereas the other nonneoplastic site had evidence of MIN at only one locus (LT-UC 13; Table 5).

Discussion

MIN is a marker of genome-wide mutations and is usually described in malignant or premalignant tissue (1–5). Only a few studies have been performed to evaluate the role of MIN in nonneoplastic tissue. MIN has been described in the nonneoplastic tissues from some HNPCC patients (10), and we recently identified it in pancreatitis without neoplasia (6). We now report the presence of MIN in the nonneoplastic but chronically inflamed mucosa of UC.

In the present investigation of LT-UC patients, we found MIN not only in a large percentage of patients with cancer and HGD, as expected, but also in patients whose mucosa was histologically negative for dysplasia or cancer. Of nonneoplastic samples tested, 50% had MIN at two or more loci, whereas none of the ischemic or infectious colitis controls had evidence of MIN (Table 4; $P \leq 0.03$). Furthermore, 287 samples from normal colonic tissue showed no evidence of MIN (9). These results indicate that MIN can occur in the chronic inflammatory setting of UC in the absence of histological evidence of dysplasia or cancer.

Extensive UC of duration greater than 8 years has long been noted to be associated with an increased risk for colorectal cancer. However, these UC-associated cancers differ in substantial ways from sporadic colon cancer. UC patients develop cancer at a younger age and not uncommonly develop multiple tumors. Furthermore, dysplasia in UC may be focal, regional, or diffuse and is often present in mucosa that appears normal at colonoscopy (10). In contrast, the adenoma that precedes a sporadic colon cancer is localized, produces a visible mass, and is not associated with widespread neoplastic change in the remaining mucosa.

Our previous studies suggest that UC cancers may also have important genetic differences from sporadic colon cancers. Aneuploidy and p53 mutations may be relatively widespread in UC and may occur in mucosa that is histologically negative for dysplasia or cancer (11). By contrast, in sporadic colon cancer, both aneuploidy and p53 mutations occur later, are confined to neoplastic tissue, and are not present in adjacent normal-appearing mucosa (12). Thus, the wide-

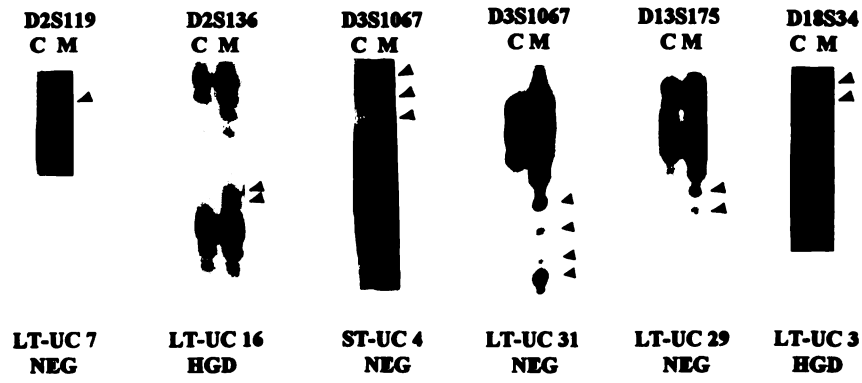

Fig. 1. MIN detected in UC DNA. The microsatellite loci are noted at the top of each tested pair. *C*, constitutional DNA (from muscularis or lymphocytes); *M*, mucosal DNA (microdissected from paraffin sections of colonic mucosa); *arrows*, microsatellite alterations. The patient number and the histological diagnosis of the patient are listed below each test pair.

Table 2 *MIN in ST-UC*

Patient	Duration (years)	MIN	Sites + for MIN
ST-UC 1	1	2 of 7	*D6S87, D17S787*
ST-UC 2	3	1 of 10	*D5S107*
ST-UC 3	3	1 of 8	*D17S261*
ST-UC 4	1	4 of 9	*D2S123, D3S1067, D6S87, D8S255*
ST-UC 5	3	1 of 7	*D6S87*
ST-UC 6	2	0 of 6	None

Table 3 *MIN in ischemic colitis or infectious colitis*

Patients	MIN
IC-1	0 of 6
IC-2	0 of 6
IC-3	0 of 7
IC-4	0 of 6
IC-5	0 of 6
IC-6	0 of 6
IC-7	0 of 6

spread, multifocal nature of the histological and genomic abnormalities in the UC colon suggests that neoplastic progression here differs significantly from that of sporadic colon cancer, and that it may be the result of genetic instability over large regions of the colon. In this context, it was interesting to find that MIN could be widespread in some of the UC patients. Three of the four LT-UC patients with HGD/cancer had MIN in histologically negative tissue remote from the site of cancer or dysplasia.

Is it possible that these UC patients represent a variant of HNPCC, a syndrome in which nonneoplastic tissue may demonstrate MIN as a result of defects in mismatch repair genes? Family histories were available from four of the seven UC patients (two ST-UC and two LT-UC) whose tissues were histologically negative and demonstrated MIN at two or more sites. None of these patients had a first-degree relative with colon cancer. These data would suggest that UC patients are probably not "variants" of the HNPCC phenotype, and furthermore, the lack of hereditary cancers in these UC patients supports the concept of an intact constitutional DNA mismatch repair system. If the DNA mismatch repair system is intact, it follows that it must be unable to fully compensate for DNA damage under certain conditions.

We hypothesize that chronic inflammation may play an important role in the development of MIN in the nonneoplastic mucosa of UC patients. Even in ST-UC, there are histological changes in the colon indicative of chronic inflammation, including aberrant crypt architecture and basal lymphoplasmacytosis. At the molecular level, inflammation produces large quantities of reactive oxygen species, including hydroxyl radicals (·OH), hydrogen peroxide (H_2O_2), and superoxide radicals (O_2^-). In the presence of reactive oxygen species, not only is DNA directly damaged, but such conditions may result in a conformational change in the DNA template that prevents accurate replication by DNA polymerases (13). In addition, heme iron from microscopic hemorrhage associated with UC may exacerbate the process of mutagenesis (14). Under normal cellular conditions, oxidative stress may be responsible for as many as 10,000 DNA-damaging events/cell/day (15). Chronic inflammation may intensify oxidative stress and may eventually produce enough DNA damage to exceed the capacity of DNA repair mechanisms. Some fraction of these mutations may repeatedly escape repair and accumulate over time. Indirect evidence of oxidative damage in UC-associated neoplasia is suggested by A-G mispairs, a lesion that can be a footprint of oxidative damage and that has been demonstrated in the mutated *ras* and *p53* genes from UC patients with cancer and dysplasia (16, 17). Further indirect evidence in support of this theory is provided by the primate model of UC, in which the animals develop spontaneous colitis and colon cancer, and inflammation is significantly associated with carcinogenesis (18). Lastly, there is support for the hypothesis that the mismatch repair system is saturatable from studies performed in *Escherichia coli*, in which a defect in the proofreading exonuclease polymerase III leads to a cascade of mutations that overwhelms the capacity of the error-correction system (19).

If our hypothesis that the chronic inflammation of UC overwhelms the DNA repair mechanisms is correct, it is notable that only 50% of nonneoplastic UC patients had evidence of MIN, and not 100%. Potential explanations for not finding a higher prevalence of MIN include the relatively small number of microsatellite sites assayed (average, seven per sample), or it could be explained by a dilutional effect of normal cells in an admixture of epithelial cells that are both MIN+ and MIN−. It is also possible that MIN could be a transient event and that patients who are currently MIN− may have been MIN+ in the past and *vice versa*. In the latter scenario, MIN might be a phenotypically silent event, as long as there are MIN− stem cells and normal cellular turnover. Lastly, it seems possible that other modifying events may influence the development of instability. We have previously shown that a germline splice-site substitution in the DNA mismatch repair gene *MSH2* is significantly associated with the development of dysplasia and cancer in UC patients but not in their non-UC parents who carry the same germline substitution (20). There does not appear to be an association between MIN and the presence of the MSH2 polymorphism in this cohort of UC patients (data not shown); however, the number of patients studied is small and may be inadequate to answer this question. Other mitigating factors that might influence the development of MIN at the genetic level include the action of modifying genes. For example, the *p53* tumor suppressor gene acts at the G_1 checkpoint to delay entry of genetically damaged cells into S phase until their DNA can be repaired (21). However, *p53* can be functionally destabilized under oxidative conditions, and the loss of the G_1 checkpoint could lead to the proliferation of cells with DNA damage (22). In addition, environmental factors might influence the development of MIN, including exposure to carcinogens or, alternatively, to antioxidants.

Table 4 *Prevalence of MIN in LT-UC, ST-UC, and controls*

	MIN ≥ 1 loci	MIN ≥ 2 loci	*P* value
LT-UC			
Negative	6 of 10 (60%)	5 of 10 (50%)	
HGD	11 of 13 (85%)	6 of 13 (46%)	
Cancer	2 of 5 (40%)	2 of 5 (40%)	
			1.0[a]
ST-UC			0.03[b]
Negative	5 of 6 (83%)	2 of 6 (33%)	
Ischemic or Infectious colitis	0 of 9	0 of 9	

[a] *P* value: LT-UC negative *versus* ST-UC negative.
[b] *P* value: LT-UC negative *versus* ischemic or infectious colitis.

Table 5 *MIN at sites negative for dysplasia from UC patients with HGD or cancer.*

Patient	Site 1 (HGD or cancer)	Site 2 (Negative)	Site 3 (Negative)
LT-UC 4	2 of 8[a] *(D5S107, D5S346)*	2 of 8 *(D2S136, D3S1067)*	2 of 8 *(D2S136, D5S107)*
LT-UC 13	5 of 10 *(D2S123, D2S136, D2S119, D13S175, D18S34)*	2 of 8 *(D2S136, D3S1067)*	1 of 8 *(D2S136)*
LT-UC 16	3 of 8 *(D2S136, D3S1067, D5S346*	0 of 7	0 of 7
LT-UC 28	3 of 10 *(D2S136, D17S261, D6587)*	3 of 9 *(D2S123, D3S1067, D8S255)*	2 of 9 *(D2S123, D8S255)*

[a] Number of loci positive for MIN; positive loci are listed in parentheses.

In the present study, neither the extent nor the duration of disease appeared to be directly associated with the development of MIN (Tables 1–4; differences not significant). The 8-year criterion for separating ST-UC from LT-UC is based on the clinical observation that the risk of cancer in UC begins to rise after this time. However, the percentage of MIN in nonneoplastic mucosa from ST-UC patients approached that of LT-UC patients (33% *versus* 50%, respectively). Importantly, both subsets of UC patients (LT-UC and ST-UC) have similarly intense chronic inflammation, which is not present in ischemic or infectious colitis. Thus, chronic inflammation alone may lead to MIN, regardless of disease duration. The increased risk of colonic neoplasia associated with the LT-UC patients may be the result of the longer time interval providing more opportunities for additional genetic damage ("second hits") with resulting neoplastic progression.

In conclusion, we report MIN in nonneoplastic colonic mucosa from patients with chronic UC, whereas colonic mucosa from control patients has no evidence of MIN. We speculate that the chronic inflammation associated with UC can lead to saturation of the DNA repair mechanism and to the subsequent accumulation of mutations over time. We have previously detected MIN in pancreatitis. These data are of interest because they suggest a mechanism of silent infidelity by which some chronic inflammatory diseases might predispose to cancer.

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