

Microsatellite Instability in Ulcerative Colitis-associated Colorectal Dysplasias and Cancers¹

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Abstract

Microsatellites are short nucleotide repeat sequences present throughout the human genome. Alterations of microsatellites, comprising extra or missing copies of these sequences, have been termed microsatellite instability. This abnormality occurs in sporadic and hereditary adenocarcinomas of the proximal colon, as well as in many other tumor types. We determined whether microsatellite instability occurred in ulcerative colitis-associated cancers or precancerous dysplasias. Sixty-three patients were evaluated, consisting of 188 samples of genomic DNA (63 normal controls, 68 cancers, 52 dysplasias, and 5 adjacent tissues) at loci D2S119, D2S123, D2S147, D10S197, and D11S904. Multiplex polymerase chain reaction was performed using one radiolabeled nucleotide, and the products were electrophoresed on denaturing polyacrylamide gels. Seventeen of the 63 patients (27%) possessed lesions showing instability at 1 or more loci. Fourteen of 68 tumor samples (21%) and ten of 52 dysplasias (19%) displayed instability. There was no tendency for a greater number of loci to manifest instability in more advanced lesions. Neither anatomic location nor loss of heterozygosity at the *p53* locus were associated with microsatellite instability by 2-way table analysis. These data support a role for defective DNA repair in the generation of a subset of both early and advanced ulcerative colitis-associated colorectal neoplastic lesions.

Introduction

Microsatellites are short repeated nucleotide sequences interspersed throughout the human genome (1, 2). The repeating unit comprising a microsatellite can be as short as one or two nucleotides; in fact, the most common type consists of dinucleotide repeats. In a subset of colorectal and other tumors, errors in DNA repair occur resulting in altered DNA length at these regions (3-5).

In HNPCC³ and in some sporadic carcinomas of the proximal colon, mutations in DNA repair genes such as the *hMSH2* or human *mutS* gene homologue, located on chromosome 2p, result in a "mutator" phenotype characterized by genetic errors at numerous genomic loci (6-9). *hMSH2* does not undergo loss of heterozygosity in the tumors of these patients (3). However, germline mutations impairing its DNA repair function occur in HNPCC patients (6-8); similarly, somatic (acquired) mutations in this gene occur in some sporadic colon cancers (3-5, 7-9). A second human DNA repair gene, located on chromosome 3p, has also been isolated (human *MLH1*); germline mutations in this gene account for a significant proportion of HNPCC cases (10, 11).

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³ The abbreviations used are: HNPCC, hereditary nonpolyposis colorectal cancer; PCR, polymerase chain reaction; UC, ulcerative colitis; LOH, loss of heterozygosity.

Microsatellite instability has now been reported in colorectal carcinoma (3-5, 12-14), gastric carcinoma (12, 15-17), pancreatic carcinoma (15), endometrial carcinoma (12, 18), non-small cell lung carcinoma (19), small cell lung carcinoma (20), prostatic carcinoma (21), and less frequently in cancers of the bladder (22), breast (23), liver, and ovary (15). The highest frequency of microsatellite instability described in a sporadic tumor type was reported for prostate cancer (21). There appears to be some variation in the prevalence of microsatellite instability according to primary tumor site. For example, instability is more frequent in cancers of the proximal colon than in distal colorectal carcinomas (4, 5). Similarly, it is very common in gastric cancers (15-17) but rare in bladder cancers (22). In some tissue types, instability predominates in poorly differentiated tumors (13, 15). Moreover, all six bladder cancers showing microsatellite instability in one study were early-stage cancers, suggesting that this type of alteration can occur early in tumorigenesis (22); 2 of 16 gastric cancers showed instability in adjacent precancerous dysplasia (16); and microsatellite instability has been shown to occur early in both *in vitro* and *in vivo* studies of colorectal tumorigenesis (14). We have found that microsatellite instability occurs early in Barrett's-associated esophageal neoplasia (24). Thus, this type of molecular alteration may constitute an early event in human malignant diseases.

We therefore reasoned that it would be logical to look at early and advanced neoplastic lesions associated with the premalignant disease, ulcerative colitis.

Materials and Methods

Tissues and DNAs. Assays were performed on normal control and premalignant or malignant tissues from 63 patients, comprising 188 samples of genomic DNA (63 normal controls, 68 cancers, and 52 dysplasias). All dysplasias were non-polypoid; DALMs were nonpolypoid raised lesions, while flat dysplasias were not visible to the naked eye. All tissues were obtained at surgical resection. Genomic DNA was extracted using standard protocols (25). Normal control DNA for each patient was obtained from histologically normal lymph nodes or normal ileal mucosa. In five cases showing microsatellite instability, adjacent tissue was analyzed. These 5 adjacent tissues consisted of 3 dysplasias adjacent to the tumor (1 mixed low/high grade, 2 indefinite) and 2 adjacent histologically normal specimens (1 normal tissue adjacent to the tumor, 1 normal tissue adjacent to the dysplasia). In all cases, microdissection was performed from paraffin blocks, resulting in neoplastic cell enrichment to a level of 70% or greater (25).

Microsatellite Instability. DNA from normal, cancerous, or dysplastic colorectal mucosa was PCR-amplified at microsatellite repeat loci *D2S119*, *D2S123*, *D2S147*, *D10S197* and *D11S904*, loci showing relatively high instability rates in colorectal tumors (3). We employed multiplex PCR (more than one locus amplified simultaneously in one reaction tube; Ref. 16). Conditions consisted of 33 cycles at 95°C ' 50 s, 58°C ' 90 s, and 72°C ' 90 s. PCR was performed using 0.2 μCi of ³²P-labeled dCTP incorporated into a 10-μl reaction mixture. PCR products were denatured in 95% formamide and electrophoresed on denaturing 6% polyacrylamide

gels, then visualized by autoradiography. Instability was defined as the presence of bands in neoplastic DNA that were not visible in corresponding normal DNA. A positive case was defined as one showing instability at one or more loci which was confirmed in two independent, separately performed PCR assays from genomic DNA.

Results

Seventeen of the 63 patients (27%) showed instability at one or more loci. Fourteen of 68 tumor samples (21%) and 10 of 52 dysplasias (19%) manifested instability. To determine the stage of neoplastic lesion at which microsatellite instability occurred, we also analyzed 5 areas adjacent to dysplasia or tumor. These 5 adjacent tissues consisted of 3 dysplasias adjacent to tumor (1 mixed low/high grade, 2 indefinite) and 2 adjacent histologically normal specimens (1 normal tissue adjacent to tumor, 1 normal tissue adjacent to dysplasia). In one dysplasia, different areas of the same lesion showed distinctive instability patterns (Table 1, specimens H4-D2 and H4-D3). Examples are displayed in Fig. 1; positive results are summarized in Table 1.

Ten lesions showed microsatellite instability at multiple loci, while 15 manifested this abnormality at only 1 locus (Table 1). Within the multiple loci-positive group, there were four dysplasias and six cancers; in the single locus-positive group, there were seven dysplasias and eight cancers. Both high-grade and low-grade dysplasias were present in each group. Dukes' stages were also represented in similar proportions in the two groups.

Tumors in the proximal colon showed a slightly higher percentage (6 of 25 samples, or 24.0%) of microsatellite instability than those in the left colon (6 of 40 samples, or 15%). However, this difference was not significant by χ^2 analysis. Among the loci studied, instability was

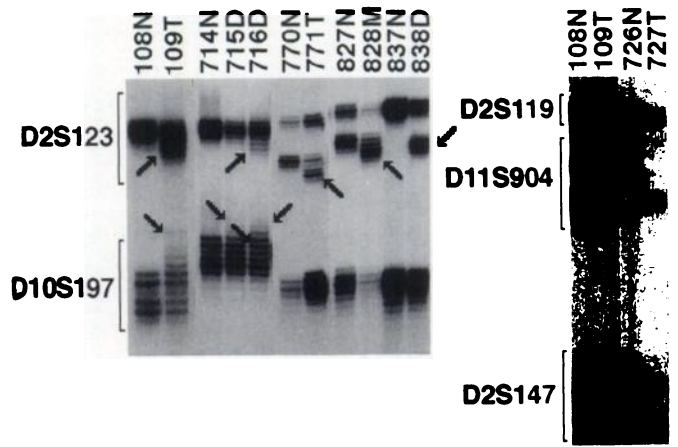


Fig. 1. Microsatellite instability detected in ulcerative colitis-associated colorectal dysplasia and cancers. *N*, normal; *D*, dysplasia; *T*, tumor; and *M*, metastatic lymphnode. Case numbers are shown above the lanes. Arrows, microsatellite instability. Left panel, loci *D2S123* on chromosome 2 and *D10S197* on chromosome 10; right panel, loci *D2S119*, *D2S147* on chromosome 2, and *D11S904* on chromosome 11.

present most often at *D2S123* (14 of 124 samples, or 11.3%). The other loci were equally represented at a lower frequency (4–8%). In agreement with published studies of colorectal cancer, instability was often widespread involving multiple genomic sites (3–5).

We previously analyzed LOH at the *p53* locus and *p53* mutations in patients with ulcerative colitis (26). These data, combined with the current data on microsatellite instability, showed no significant correlation by 2-way table analysis (Fisher's exact test; Table 2).

Table 1 Lesions showing microsatellite instability

DNA	Histology ^a	Location	<i>D2S119</i>	<i>D2S123</i>	<i>D2S147</i>	<i>D10S197</i>	<i>D11S904</i>
Multiple loci-positive lesions							
716D	DALM	L ^b	N	P	N	P	P
838D	Flat HGD	NA ^c	P	P	N	N	N
851D	Dukes C2 LP	L	P	P	N	N	P
H4-D3	LGD-HGD, area 2	L	N	P	P	P	P
109T	Dukes' A pooled DALM	R	N	N	N	P	P
657T	Dukes' D ADCA	L	P	P	P	P	P
727T	Dukes' B1 ADCA	R	P	P	P	P	P
828M	Dukes' D metastatic LN PDSR CA	NA	P	P	P	N	N
843T	Dukes' C2 PDSR CA	R	P	P	N	N	P
H16T	Dukes' C2 MDCA	R	P	N	N	P	N
Single locus-positive lesions							
715D	DALM	R ^d	N ^e	N	N	P	N
760D1	DALM	R	N	N	N	N	P
761D2	DALM	L	N	N	N	N	P
773D	Flat HGD	R	P ^f	N	N	N	N
780Dd	DALM	L	N	P	N	N	N
839D	DALM	NA	N	P	N	N	N
H4-D2	LGD-HGD, area 1	L	N	N	N	P	N
126T	Dukes' C2 MDCA	L	N	N	N	N	P
694T	Dukes' B1 ADCA	R	NA	P	NA	NA	NA
862T	Dukes' B1 MDCA	L	N	P	N	N	N
733T	Dukes' B1 WDCA	P	N	N	N	P	N
762T	Dukes' C2 WDCA	L	NLOH ^g	N	P	N	N
771T	Dukes' B1 WDCA	L	N	P	N	N	N
823T	Dukes' D Metastatic CA	NA	N	P	N	N	N
H21T	Dukes' C2 Mucinous	L	N	N	N	P	N

Chromosomal loci are listed at top.

^a DALM, dysplasia-associated lesion or mass; HGD, high grade dysplasia; LP, linitis plastica; LGD, low grade dysplasia; ADCA, adenocarcinoma; LN, lymph node; PDSR, poorly differentiated signet ring cell adenocarcinoma; MDCA, moderately differentiated carcinoma; WDCA, well differentiated adenocarcinoma.

^b Left colon.

^c Not available.

^d Right colon.

^e Negative for microsatellite instability.

^f Positive for microsatellite instability.

^g Negative for instability, but positive for loss of heterozygosity.

Table 2 Relationship between LOH at *p53* locus and microsatellite instability^a

	LOH at <i>p53</i> locus	
	Positive lesions	Negative lesions
Microsatellite instability		
Positive lesions	3	4
Negative lesions	23	27

^a *P* = not significant by Fisher's exact test.

Discussion

Our data indicate that microsatellite instability occurs in approximately one-fourth of patients with ulcerative colitis-associated dysplasia and carcinoma. This overall frequency is similar to that reported in sporadic colorectal cancer (3–5). In our study, instability occurred as frequently in dysplasias as it did in carcinomas. Moreover, there was no difference between the multiple loci-positive and single locus-positive cases in terms of the stage of the lesion. *i.e.*, there was no apparent tendency for more advanced lesions to show involvement of multiple loci. These data suggest that microsatellite instability occurs in premalignant tissues, at the dysplastic stage, in at least a subset of patients with ulcerative colitis; these findings also show that microsatellite instability can occur in flat or raised nonpolypoid dysplastic lesions as well as in the polypoid adenomas and carcinomas of sporadic colon cancer patients. We were unable to demonstrate instability in normal tissues adjacent to dysplasias or cancers, implying that microsatellite instability does not necessarily give rise to the clone that progresses to dysplasia or cancer.

We observed a slight predominance of microsatellite instability in the proximal *versus* the distal colon, which also resembles sporadic colon cancer (3–5). A recently published abstract by Hamelin *et al.*, described 48 sporadic colorectal cancers studied for microsatellite instability at 100 chromosomal loci (27). None of the left-sided cancers showed instability, whereas 37% of the right-sided cancers were positive for this abnormality.

Microsatellite instability tended to occur most often at *D2S123*, although this tendency was not statistically significant. The *D2S123* locus is closely linked to the *hMSH2* gene responsible for HNPCC (7). Microsatellite instability in HNPCC patients is due to germline mutations in *hMSH2*, *hMLH1*, and possibly other DNA repair genes. We speculate that mutations in these genes may be related to carcinogenesis in patients with ulcerative colitis. However, a well-defined hereditary predisposition to cancer has not yet been as convincingly shown in UC as in HNPCC. Cancer developing in UC patients is presumed to be related to chronic inflammation rather than to hereditary factors. Therefore, it is somewhat surprising to find microsatellite instability so commonly in these patients. If *MSH2*, *MLH1*, or other DNA repair genes are involved in UC-associated carcinogenesis, mutations in these genes may occur somatically rather than in the germ line.

There was no significant relationship between LOH at the *p53* locus and microsatellite instability in ulcerative colitis-associated lesions. These findings suggest that genomic instability does not always occur in concert with *p53* inactivation; these two aberrations may describe alternate carcinogenesis pathways.

The unstable nature of the dinucleotide repeats evaluated in this and previous studies (3–5, 9, 12–21) is reminiscent of instability affecting other DNA elements. Trinucleotide repeats are particularly prone to replication infidelity (28). Classic examples of this type of genetic error include fragile X syndrome (29), spinobulbar atrophy (30), myotonic dystrophy (31), and Huntington's disease (32). All of these diseases are characterized by unstable trinucleotide repeats in germline DNA that may experience amplification from one generation to

the next. The dinucleotide instability described here and previously (3–5, 9, 12–21) appears to be of a different nature, occurring only in neoplastic tissue rather than in constitutional cells. This particular type of genetic error probably results from defective DNA repair genes located on chromosomes 2p and 3p, possibly along with other similar, as yet unidentified, genes (7–11). Other types of genomic infidelity involving repetitive DNA regions have also been described, for example, involving minisatellites composed of 10–100 nucleotide repeating units (28). In one recent report, rare constitutional alleles of the *HRAS1* minisatellite locus were associated with a high risk of developing cancer (33). This type of genetic abnormality may represent a primary defect, rather than a secondary manifestation of mutation in a DNA repair gene.

Only five chromosomal loci were examined in this study; not all loci may show instability in any given tumor (3–5). It is possible that if more loci were examined, the percentage of positive cases would still rise further. Nevertheless, the discovery of instability in 27% of our ulcerative colitis patients suggests that defective DNA repair is important in the development of at least a subset of UC-associated neoplastic lesions.

Note Added in Proof

Kern *et al.* have now reported microsatellite instability in a series of UC-associated neoplasms (34).

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