

Microsatellite Instability in Aberrant Crypt Foci from Human Colons¹

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Abstract

Aberrant crypt foci (ACF) are distinct microscopic lesions of the colon thought to be the earliest identifiable precursors of colon cancer. As precursors of colon cancer, ACF may contain mutations in genes that are altered early in colorectal tumorigenesis. Candidates for these genes include *APC*, *K-Ras*, and those of the DNA mismatch repair system. Some colon cancers with mutations in DNA mismatch repair genes are characterized by genomic instability at simple repeated sequences, also known as microsatellite instability. In this study, we analyzed 19 ACF (≥ 20 crypts/focus) and adjoining, microscopically normal colonic mucosa from 10 colon cancer patients for the presence of microsatellite instability. DNA from two ACF from two different patients displayed microsatellite instability. None of the DNA samples from normal mucosa displayed microsatellite instability. These observations support the role of ACF as a precursor to colon cancer and provide some evidence that mutations in DNA mismatch repair genes are early somatic events in colon cancer.

Introduction

CRC³ develops through a series of distinct histological stages that progress from normal mucosa to premalignant adenomatous polyps to invasive cancer (1). Previous studies have identified and characterized microscopic lesions called ACF in whole-mount preparations of colon from rodents treated with carcinogens (2-5). The ACF may be a distinct histological lesion that precedes the development of an adenoma and therefore the earliest identifiable precursor of colon cancer (3, 6, 7). ACF are composed of clusters of abnormally large, dark-staining, slightly elevated mucosal crypts. Recent studies have reported an increased frequency of ACF in the colonic mucosa of patients with CRC when compared to the mucosa from patients without CRC (7). In addition, *K-ras* and *APC* mutations have been identified in colonic ACF from colon cancer patients (8-10). These observations suggest that ACF are microscopic lesions with the potential to progress to colonic neoplasm.

This study tests the hypothesis that ACF are histological precursors to colon cancer by analyzing ACF for microsatellite instability, a marker for defective DNA mismatch repair (11-13). Approximately 20% of all colon cancers are characterized by microsatellite instability (14-16), a marker for a mutator phenotype that leads to the accumulation of a high frequency of somatic mutations (17). Studies by Shibata *et al.* (18) have shown that microsatellite instability is present in microscopic adenomas. Thus, one might predict that microsatellite instability will also be present in a subset of ACF.

We have analyzed 19 colonic ACF and adjoining microscopically normal colonic mucosa from 10 colon cancer patients for microsatellite instability. Microsatellite instability has been observed in two ACF from two different patients. Additionally, LOH was observed in one ACF at a locus on 18q that maps close to the *DCC* and *DPC4* genes.

Materials and Methods

Patient Samples. Specimens of overtly normal-looking colonic mucosa were obtained from cancer patients who underwent resection for colon cancer at the George Washington University Hospital. The normal-looking colonic mucosa (mean area, 10 cm²) was sampled at a point approximately 5 cm from the colon tumor. Specimens of normal-looking colonic mucosa were located in the proximal colon for patients I, II, III, V, VIII, and X and in the distal colon for patients IV, VI, VII, and IX.

Microscopic Analysis. For the detection of ACF, colonic mucosa was stained with 0.2% methylene blue and examined under a light microscope (4). ACF composed of 20 or more crypts were microdissected from the colonic mucosa and stored in separate tubes. Nineteen ACF from 10 different patients were collected as well as a normal sample from each patient. The samples were stored at -70°C for subsequent DNA extraction (4).

Microsatellite Instability. DNAs were amplified by the PCR at loci containing polymorphic microsatellite repeats: *D5S346*, *D7S471*, *D7S473*, *ACTC*, *D17S261*, *D18S47*, *BAT26*, and *BAT40*. PCR conditions consisted of 35 cycles at 95°C (60 s), 52-58°C (60 s), and 72°C (60 s). PCR was performed using 1.5 mM MgCl₂, 1 mM of the primer pair at each microsatellite repeat locus, 0.25 mM of upstream primer labeled with 3 mCi of [³²P]ATP, 3.2 mM deoxynucleotide triphosphates, 3% DMSO, and 1.25 units *Taq* polymerase (Boehringer Mannheim Co., Indianapolis, IN) in a 25-ml reaction. PCR products were electrophoresed on denaturing 7% polyacrylamide (19:1) gels (National Diagnostics, Atlanta, GA) and visualized by autoradiography.

Results and Discussion

DNA from 19 ACF samples (≥ 20 crypts/focus) from 10 different patients with colon cancer were analyzed at 8 microsatellite repeat loci. A normal sample from each of the 10 patients was also examined as a control. The results of this analysis are summarized in Table 1. The majority of samples generated identical microsatellite patterns as the normal specimen, with the exception of two lesions. One sample, designated Patient III-6 ACF, displayed instability at six of eight loci tested. A second sample, designated Patient VIII-19 ACF, displayed instability at four of eight loci. Examples of this instability are seen in Fig. 1a and b.

In addition to evaluating microsatellite instability, this assay was used to observe LOH at particular loci. In Patient VI, only one allele instead of two seemed to be present at the *D18S47* locus when the sample 14 ACF was compared to the corresponding normal sample (Fig. 2). This suggested the possibility of a LOH event at the *D18S47* locus, a marker on chromosome 18q adjacent to the candidate tumor suppressors *DCC* and *DPC4*.

This is the first study that compares colonic ACF and adjoining microscopically normal colonic mucosa from colon cancer patients to detect the presence of microsatellite instability. These results demonstrate the presence of microsatellite instability in colonic ACF from 2

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³ The abbreviations used are: CRC, colorectal cancer; ACF, aberrant crypt foci; LOH, loss of heterozygosity.

Table 1 Number of alleles identified at each dinucleotide repeat loci for 19 different ACF samples

Dinucleotide repeat loci were assayed by PCR to determine the number of alleles present at a particular marker for these ACF patient samples. PCR products were visualized by autoradiography. The presence of more than two distinct alleles was scored as unstable. All normal samples displayed two alleles.

Sample/marker	D5S346	D7S471	D7S473	ACTC	D17S261	D18S47	BAT26	BAT40
I-2ACF	2	2	2	2	2	2	2	2
II-4ACF	2	2	2	2	2	2	2	2
III-6ACF	3	3	2	4	3	2	4	4
IV-8ACF	2	2	2	2	2	2	2	2
IV-9ACF	2	2	2	2	2	2	2	2
V-11ACF	2	2	2	2	2	2	2	2
V-12ACF	2	2	2	2	2	2	2	2
VI-14ACF	2	2	2	2	2	1 ^a	2	2
VI-15ACF	2	2	2	2	2	2	2	2
VII-17ACF	2	2	2	2	2	2	2	2
VIII-19ACF	3	3	4	2	3	2	2	2
VIII-20ACF	2	2	2	2	2	2	2	2
IX-22ACF	2	2	2	2	2	2	2	2
IX-23ACF	2	2	2	2	2	2	2	2
X-25ACF	2	2	2	2	2	2	2	2
X-26ACF	2	2	2	2	2	2	2	2
X-27ACF	2	2	2	2	2	2	2	2
X-28ACF	2	2	2	2	2	2	2	2
X-29ACF	2	2	2	2	2	2	2	2

^a LOH on 18q.

of 10 patients. Microsatellite instability is more commonly detected in carcinomas of the proximal colon (14–16), and although the number of cases studied in this experiment is relatively small, it is interesting to note that in patients III and VIII, the ACF were located in the proximal colon. The ACF from patient VI with LOH at 18q was located in the distal colon. Although microsatellite instability is more common in cases of hereditary nonpolyposis CRC, this information was unavailable in either of the patients whose ACF showed microsatellite instability.

These results also suggest that there is heterogeneity among colonic ACF from the same patient. In patient VIII, two different ACF were

evaluated, however, only one lesion displayed instability. Such heterogeneity among ACF from the same patients has been observed in previous studies when ACF were analyzed for mutations in *K-ras* and *APC* (8–10). In combination with this past research, these studies suggest the independent origin of these lesions. Experiments are currently underway to analyze ACF with and without microsatellite instability for the presence of mutations in *K-ras*, *APC*, and *p53* genes to further evaluate the idea that the genetic targets of mutation are different in the colonic lesions with microsatellite instability than in lesions without instability (19). In conclusion, our observations support the role of ACF as a precursor to colon cancer and provide evidence that suggests that mutations in the DNA mismatch repair genes are early somatic events in colon cancer.

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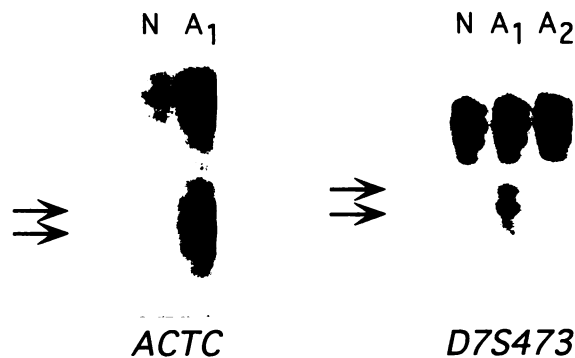


Fig. 1. Microsatellite instability detected in ACF samples from two colon cancer patients. a, DNA from a normal (N) and an ACF (A_1) sample of patient III. b, DNA from a normal (N) sample and two ACF samples (A_1 and A_2) of patient VIII. →, instability.



Fig. 2. LOH observed in an ACF sample from colon cancer patient VI. DNA from a normal (N) sample and two ACF samples (A_1 and A_2) of patient VI. LOH occurred in Lane A_1 .

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