

# Microsatellite Instability Occurs Frequently and in Both Diploid and Aneuploid Cell Populations of Barrett's-associated Esophageal Adenocarcinomas<sup>1</sup>

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## Abstract

Alterations of microsatellites consisting of extra or missing copies of these sequences occur at relatively high frequencies in sporadic and hereditary colorectal adenocarcinomas, gastric and pancreatic cancers, and at lower frequencies in endometrial, bladder, ovarian, and other carcinomas. We determined the prevalence of microsatellite instability in esophageal adenocarcinoma, Barrett's esophagus, and squamous cell carcinoma of the esophagus. Assays were performed on 105 patients, including 28 subjects with Barrett's metaplasia, 36 with Barrett's-associated adenocarcinoma, and 42 with primary esophageal squamous cell carcinoma. Flow cytometric nuclear sorting based on DNA content was performed on 25 of the adenocarcinomas prior to DNA extraction. Specimens from 11 of the 106 patients (10%) showed instability at 1 or more chromosomal loci. Instability was seen in 2 of 28 patients (7%) with Barrett's metaplasia alone, in 8 of 36 (22%) with adenocarcinoma, and in 1 of 42 (2%) with squamous cell carcinoma. Among the 25 flow cytometrically sorted adenocarcinomas, instability occurred in 8 (32%); sorted diploid nuclei from these tumors showed instability in 4 of 8 cases (50%). These data indicate that microsatellite instability occurs frequently in Barrett's-associated esophageal adenocarcinoma. They also suggest that in esophageal adenocarcinomas, microsatellite instability can develop as an early event in metaplasia and in diploid tumor cells, before aneuploidy occurs.

## 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 completely different lengths of DNA at these regions (3–5).

In HNPCC<sup>3</sup> and some sporadic cancers of the proximal colon, mutations in DNA repair genes, such as the *MSH-2* or human *mutS* gene homologue located on chromosome 2p, result in a "mutator" phenotype characterized by genetic errors at numerous genomic loci (6–9). *MSH-2* is not a tumor suppressor gene; it does not suffer loss of heterozygosity in the tumors of these patients (3). It undergoes a germline mutation which impairs its DNA repair function in HNPCC patients (6–8); similarly, a somatic (acquired) mutation in this gene probably occurs in the tumors of some sporadic colon cancer patients (3–5, 7–9). A second human DNA repair gene, located on chromo-

some 3p, has also been isolated (human *MLH-1*); germline mutations in this gene, too, account for a significant number of HNPCC cases (10, 11).

There appears to be 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, instability is very common in gastric cancers (12–14) but rare in bladder cancers (15).

## Materials and Methods

**Tissues and DNA.** Assays were performed on normal control and premalignant or malignant tissues from 106 patients, including 28 with Barrett's metaplasia alone, 36 with Barrett's-associated adenocarcinoma, and 42 with esophageal squamous cell carcinoma. Tissues were obtained at surgery or endoscopy and frozen in liquid nitrogen. Loci *D2S123*, *D10S197*, *D2S147*, *D2S119*, and *D11S904*, which frequently show microsatellite instability in colorectal carcinoma, were examined in this study. Normal control DNA for each patient was obtained from peripheral blood leukocytes or normal gastric mucosa. Flow cytometric nuclear sorting based on DNA content was performed on 25 of the adenocarcinomas prior to DNA extraction (16). In all 25 of these cases, DNA was analyzed from normal tissue and from sorted diploid and aneuploid cell populations in the cancers. All 28 Barrett's metaplasia biopsies were processed *en bloc*. Genomic DNA was extracted via standard protocols (17).

**Microsatellite Instability.** The DNA of normal, tumor, or Barrett's mucosa was amplified by PCR at microsatellite repeat polymorphisms *D2S123*, *D2S147*, *D2S119*, *D10S197*, and *D11S904*, loci showing instability rates ranging from 67 to 100% in tumors that manifest this abnormality (3). We used multiplex PCR (more than one locus amplified simultaneously in one reaction tube) (13). PCR conditions consisted of 33 cycles at 95°C for 50 s, 58°C for 90 s, and 72°C for 90 s. PCR was performed using 0.2 μCi of [<sup>32</sup>P]dCTP incorporated into a 10-μl reaction mixture. PCR products were denatured in 95% formamide, electrophoresed on denaturing polyacrylamide gels, and then visualized by autoradiography. Instability was defined as the presence of bands in tumor DNA that were not visible in corresponding normal DNA.

## Results

Specimens from 11 of the 106 patients (10%) showed microsatellite instability at 1 or more chromosomal loci. Instability was seen in 2 of 28 patients (7%) with Barrett's metaplasia, in 8 of 36 (22%) with adenocarcinoma, and in 1 of 42 (2%) with squamous cell carcinoma. Examples are displayed in Fig. 1.

Among the 25 flow cytometrically sorted adenocarcinomas, instability occurred in 8 (32%). The diploid component of the tumor showed instability in 4 of these 8 positive cases (50%). Moreover, in three of the four tumors manifesting instability in diploid cells, additional secondary microsatellite alterations were observed in aneuploid tumor DNA. Positive results obtained at the five loci are summarized in Table 1. Instability was present at comparable frequencies among the various loci. In agreement with published studies of colorectal

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<sup>3</sup> The abbreviations used are: HNPCC, hereditary nonpolyposis colorectal cancer; PCR, polymerase chain reaction.

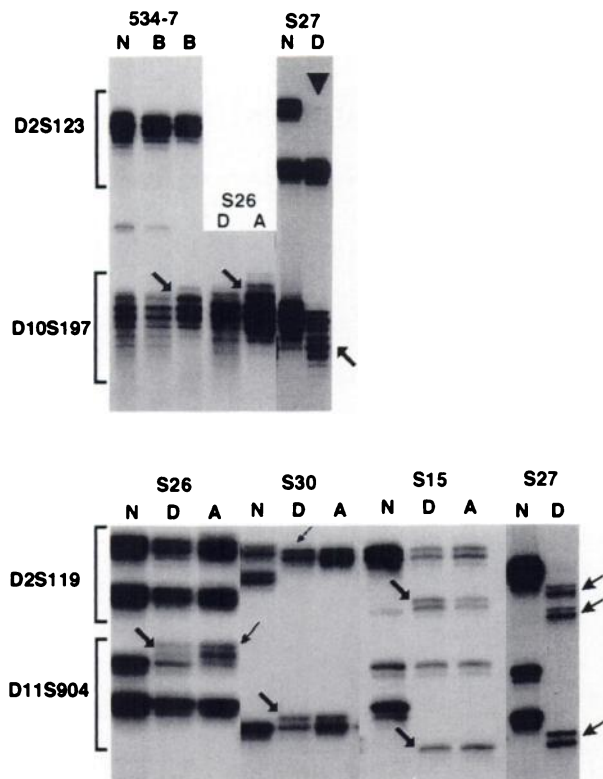


Fig. 1. Microsatellite instability detected in esophageal carcinoma and Barrett's esophagus. N, normal DNA; D, diploid tumor DNA fraction; A, aneuploid tumor DNA fraction; B, DNA from Barrett's metaplasia. Ordinate, loci assayed. Patient numbers are listed above tissues. Angled arrows, instability; arrowhead, loss of heterozygosity. Note instability in diploid cell DNA of tumors S27, S26, S30, and S15. Also note secondary instability in aneuploid cell DNA of tumor S26.

cancer, instability was often widespread, involving multiple genomic sites (3-5).

**Discussion**

Esophageal cancer shares molecular pathophysiological features with colorectal and gastric carcinomas. These tumors are characterized by frequent loss of heterozygosity affecting chromosomes 5 and 17 (18-24). They also show high rates of p53 mutation (25-28). Our results confirm that these three tumor types share another molecular abnormality, microsatellite instability. These findings support the

hypothesis that the frequency of instability varies according to tissue of origin (3-5, 7-14, 29-34) because much higher rates were found in adenocarcinoma than in squamous cell carcinoma of the esophagus. Microsatellite instability has now been reported in colorectal (3-5, 29, 33, 34), gastric (12-14, 29), pancreatic (12), endometrial (29, 30), non-small cell lung (31), and small cell lung (32) carcinomas and less frequently in cancers of the bladder (15), breast, liver, and ovary (12). In some tumor types, instability predominates in poorly differentiated tumors (12, 33). All 6 bladder cancers showing microsatellite instability in one study were early stage cancers, suggesting that this type of alteration can occur early in tumorigenesis (15), but only 2 of 16 gastric cancers showed instability in adjacent precancerous dysplasia (13). However, microsatellite instability has been shown to occur early in both *in vitro* and *in vivo* studies of colorectal tumorigenesis (34).

Our data suggest that although it is relatively uncommon in esophageal squamous cell carcinoma, microsatellite instability occurs more frequently in Barrett's-associated adenocarcinoma. Furthermore, the observation of microsatellite instability in one patient with Barrett's metaplasia and in the sorted diploid cells of four Barrett's adenocarcinomas suggests that instability may develop as an early event in Barrett's-associated neoplastic progression. The numbers we report here may represent conservative estimates of the prevalence of this abnormality in Barrett's metaplasia, since the Barrett's biopsies were processed *en bloc*, in contrast with our flow-sorted adenocarcinomas, where a much higher rate of instability was detected. Our data are consistent with earlier studies demonstrating other forms of genomic instability in both metaplastic and dysplastic Barrett's mucosa (16, 24, 35, 36). Moreover, in the current study, some aneuploid nuclei contained secondary microsatellite alterations superimposed on primary changes present in diploid nuclei from the same tumors. This finding suggests that instability may continue through multiple stages of neoplastic progression, in addition to occurring at initiation.

Only five chromosomal loci were examined in this study; not all loci show instability in any given tumor (3-5). It is possible that if more loci were examined, the percentage of positive cases would rise further. Nevertheless, the discovery of instability in 22% of esophageal adenocarcinoma patients suggests that this mechanism is important in at least a subset of esophageal adenocarcinomas.

The unstable nature of the dinucleotide repeats evaluated in this and previous studies (3-5, 9, 12-15, 29-34) is reminiscent of instability affecting other DNA elements. Trinucleotide repeats are

Table 1 Patients showing microsatellite instability

DNA	Histology	D2S123	D10S197	D2S119	D11S904	D2S147
S1D	ADCA <sup>a</sup>	N	N	N	N	N
S1A		N	P	N	N	N
S7D	ADCA	N	N	N	N	N
S7A		N	NLOH	N	P	N
S10D	ADCA	N	N	N	N	N
S10A		N	N	P	N	N
S11D	ADCA	N	N	N	N	N
S11A		N	N	P	N	N
S15D	ADCA	N	N	P	P	N
S15A		NLOH	NLOH	NCHG	NCHG	NLOH
S26D	ADCA	N	N	N	N	N
S26A		N	N	N	P x2	N
S27D	ADCA	NLOH	P	P	P	P
S27A		NCHG	NCHG	NCHG	P x2	NCHG
S30D	ADCA	P	P	P	P	P
S30A		NCHG	P x2	NCHG	NCHG	NCHG
409B	Barrett's metaplasia	N	P	NLOH	P	NLOH
S37B	Barrett's metaplasia	N	P	N	N	N
E228	SCCA	P	P	N	N	N

<sup>a</sup> ADCA, adenocarcinoma; SCCA, squamous cell carcinoma (diploid and aneuploid tumor components are listed separately for adenocarcinomas); P, positive for microsatellite instability; P x2, secondary (additional) instability in aneuploid fraction; N, negative for microsatellite instability; NLOH, negative for instability but positive for loss of heterozygosity; NCHG, no change in aneuploid cell band pattern relative to sorted diploid cell band pattern.

particularly prone to replication infidelity (37). Classic examples of this type of genetic error include fragile X syndrome (38), spinobulbar atrophy (39), myotonic dystrophy (40), and Huntington's disease (41). 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, 12–15, 29–34) 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 (e.g., involving minisatellites composed of 10–100 nucleotide repeating units) (37). In one recent report, rare constitutional alleles of the *HRAS1* minisatellite locus were associated with a high risk of developing cancer (42). This type of genetic abnormality may represent a primary defect, rather than a secondary manifestation of mutation in a DNA repair gene.

Our data indicate that microsatellite instability occurs in approximately one-fourth of Barrett's-associated adenocarcinomas. Furthermore, the presence of instability in diploid tumor DNA, as well as in metaplastic Barrett's epithelium, supports the hypothesis that instability may sometimes occur early in Barrett's-associated neoplastic transformation. The occurrence of secondary microsatellite alterations in aneuploid DNA suggests that these abnormalities continue to accumulate as the neoplastic process progresses. Larger studies are needed to determine the stage of neoplastic progression at which microsatellite instability occurs, and whether it predisposes toward the development of cancer. Somatic or germline mutations in known DNA repair genes should be sought in tumors manifesting microsatellite instability. Future studies should also focus on possible downstream genetic victims of instability, since microsatellite instability may exert its carcinogenic effect by altering the function of specific target genes.

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