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Chromosome 18 Allele Loss at the D18S6 Locus in Human Colorectal Carcinomas

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This is the first report of chromosome 18 allele loss in colorectal carcinomas from FAP patients and concurrent allele losses on chromosomes 5 and 18 in sporadic colorectal cancer. This is based on our investigation of twenty-two colorectal carcinomas from sporadic cases and FAP patients which revealed tumor-specific allele loss of at least 44% at the D18S6 locus on chromosome 18 in informative cases. These results coupled with the tentative assignment of an HNPCC gene to chromosome 18 suggests that a gene on chromosome 18 may be involved in the etiology of some colon cancers. Possible mechanisms involving genetic changes on chromosome 18 in colon cancer are discussed in relation to tumor- or growth-suppressor genes.

Our current understanding of the molecular etiology of cancer is that tumor development and progression results from several specific genetic changes. Research on oncogenes, growth factors and their receptors offers some insight on how some genetic changes may alter normal growth control processes (reviewed in ref 1). Recently researchers have begun to study another category of genes, tumor- or growth-suppressor genes, that have been hypothesized to have an important role in the etiology of many cancer types (2,3).

Carcinogenesis in humans and laboratory animals has been described to be a multistep process (4). Colon cancer is probably a good model system to study the sequence and resulting effects of

Abbreviations: FAP, familial adenomatous polyposis; HNPCC, hereditary nonpolyposis colon cancer; RFLP, restriction fragment length polymorphism; CFS, cancer family syndrome; JK, Kidd blood group

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genetic changes that occur because several stages in the carcinogenic process are readily identified histopathologically from normal mucosa to early proliferative changes to adenoma to carcinoma (5-7).

Karyotypic studies have shown that most colorectal carcinomas are aneuploid with a variety of nonrandom chromosomal structural modifications (8). Recently, molecular biologic techniques have been used to determine the significance of these chromosomal findings in colon cancer (reviewed in ref 9). We examined normal versus tumor DNA from sporadic and FAP patients for tumor-specific allele loss on chromosome 18 because karyotypic studies have reported contrasting results on the frequency of chromosome 18 loss in colorectal cancer (8,10).

MATERIALS AND METHODS

Tumor tissue was obtained from surgical specimens from 22 colorectal carcinoma patients, including three FAP patients (11). Any gross contaminating normal stromal tissue was completely dissected away from each tumor specimen. Adjacent normal mucosa was also obtained from 14 of these patients for use as a normal control, and blood samples were used in the remaining eight patients as the normal control tissue.

Detailed family histories were obtained on the three FAP patients (nos. 22-24, see Table 1), one of whom (no. 24) had extracolonic manifestations typical of the Gardner Syndrome (manuscript in preparation). The remainder of cases were classified as sporadic because a positive family history was not reported in the patients clinical records. The known inattention to family history contained in most hospital records indicates that one cannot exclude the absence of hereditary cancer. Furthermore, one must consider the possibility of a spontaneous germline mutation, variable expressivity of the phenotype, reduced penetrance of the deleterious gene, missed paternity, and other confounders which could lead to an erroneous assumption of "sporadic".

We used the L2.7 probe for the D18S6 gene which detects a biallelic RFLP with 7.5 and 10 kb fragments in Pst I digested DNA (12,13). This probe has recently been assigned to chromosome 18 as D18S6 (13,14).

High molecular weight DNA was isolated from tumor and normal tissue (11,15). The DNA (10 ug) was digested to completion with Pst I (New England Biolabs) at 37°C overnight and electrophoresed as normal/tumor pairs on 0.8% agarose gels at 25v for 22 hours. The fractionated DNA was then transferred to Nytran membranes (Schleicher and Schuell) using the standard Southern blotting techniques (11,16). The L2.7 probe (ATCC #57305) was oligo-labelled with [alpha-^{32}P] dCTP (ICN) using a random primer kit (Boehringer Mannheim) following the manufacturer's protocol. Hybridizations were done in 25% 1M $NaPO_4$ pH 7.2, 1.46% (w/v) NaCl, 5% (w/v) SDS, 10% (w/v) PEG 6000, and 0.2% 0.5M EDTA at 65°C overnight. 0.5 ml of 10 mg/ml denatured salmon sperm DNA was added to the hybridization bag with the denatured probe (1 x 10^6 cpm/ml). The blots were washed the following day twice in 2X SSC at room temperature for 5 minutes, followed by 4x30 minute washes at 65°C in 0.5% SDS, 0.05M $NaPO_4$ pH 7.2. The blots were then exposed to Kodak XAR-5 film at -70°C for 24 hours.

RESULTS

The 22 normal/tumor DNA pairs were examined for tumor-specific allele loss at the Pst I RFLP of the D18S6 locus on chromosome 18 using the L2.7 probe. Nine patients were heterozygous at the D18S6 locus and one patient's normal DNA (no. 15) was not readable because of partial degradation of the DNA (Table 1). Of the nine informative cases, three (2 FAP and 1 sporadic carcinoma) show obvious tumor specific allele-loss (Table 1, nos. 18, 22, and 24, also see figure 1). One other informative case (sporadic carcinoma) showed strong allelic imbalance in the tumor as compared to the normal tissue (Table 1 no. 19, also see figure 1). Persistence of this weakly represented allele in the tumor sample DNA is attributed to the presence of some contaminating stromal and inflammatory cells in the tumor. In the remaining five informative cases, the allelic balance amplitude between normal and tumor DNA remained the same. The case that was not readable for the normal DNA also had a strong allelic imbalance in the tumor, which may represent another instance of allele loss with persistence of a weak allele in tumor DNA due to contaminating normal tissue (Table 1, no. 15). Excluding the latter case, the overall frequency of allele loss among this set of tumors was 4/9 (2 FAP and 2 sporadic carcinomas) at the D18S6 locus on chromosome 18.

Table 1

Alleles at the D18S6 locus in normal and colorectal carcinoma tissues

PATIENT	SITE	NORMAL	TUMOR
4	lc	2/1	2/1
6	lc	2/1	2/1
8	s	2/1	2/1
12	s	2/1	2/1
14	rs	2/1	2/1
15	lc		?/1
18	lc	2/1	2/
19	c	2/1	2/
22	rc	2/1	/1
24	r'	2/1	2/

Abbreviations: rs, rectosigmoid colon; s, sigmoid colon; lc, left colon; rc, right colon; c, colon; r', cell line derived from rectal tumor of FAP patient (Boman BM, manuscript in preparation); ?, tumor sample with less certain allele loss. Tumor and normal (adjacent normal mucosa in patients 4-15; peripheral leukocytes in patients 18-24) DNA from colorectal carcinoma patients (nos. 22, 24 had confirmed FAP) was hybridized with the L2.7 probe for the D18S6 locus. D18S6 is biallelic in human genomic DNA digested with Pst I using the L2.7 probe. "2/1" indicates heterozygosity and "2/ " or " /1" indicates homozygosity. A blank space indicates that the marker was not tested or was not readable. The cases (n=12) which were not heterozygous in the normal DNA were not included in this table.

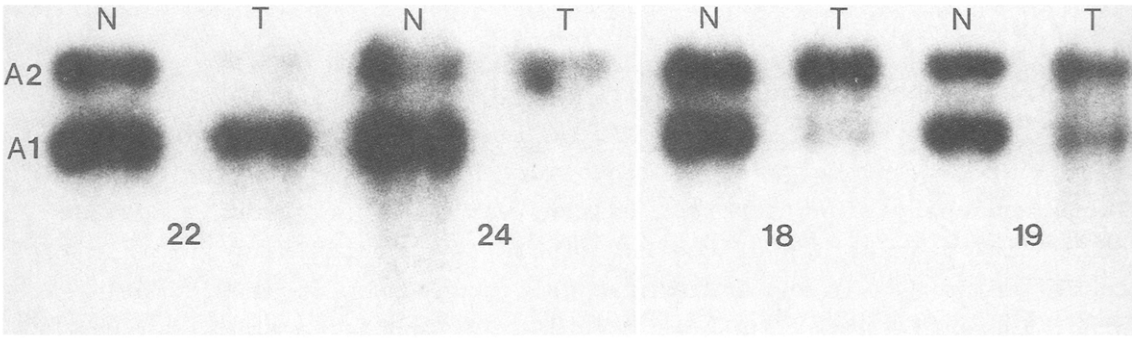

Fig. 1. Loss of alleles at the D18S6 locus on human chromosome 18 in colorectal carcinomas. Patient numbers centered below photographs refer to Table 1. DNA from matched normal (N)/tumor (T) pairs was hybridized to the ^{32}P-labelled cDNA probe L2.7 for the D18S6 locus on chromosome 18. L2.7 reveals a RFLP with fragments of 10kb ("A2") and 7.5 kb ("A1") in Pst I digested human genomic DNA. Numbers on the left denote the observed alleles.

DISCUSSION

A two-step genetic mechanism similar to that proposed for retinoblastoma has also been proposed for colorectal cancer based on the initial molecular studies (11,17). Studies that provided evidence to support this hypothesis used FAP as a model system because of its autosomal dominant mode of gene transmission and high risk for colon cancer in affected individuals who are not treated (18,19). The FAP gene has recently been mapped to the long arm of chromosome 5 (17, 20, 21), and tumor-specific loss of chromosome 5 alleles has been detected in colorectal carcinomas from some FAP patients (22). These data indicate that in FAP patients the loss of the remaining normal allele on chromosome 5 may be necessary for development of the carcinoma. It is also probable that somatic changes in this same gene occur in sporadic colorectal carcinomas because frequent (e.g. 20-40%) tumor-specific chromosome 5 allele loss has been detected in these carcinomas as well (11,22,23). This suggests that a tumor- or growth-suppressor gene is present on chromosome 5 and that mutation or loss of the two homologous copies of this gene are important events in the development of some hereditary and sporadic colorectal carcinomas.

However, the problem is probably much more complex than simply a two hit genetic mechanism based on the variety of nonrandom chromosomal changes that occur in this tumor (8,10). Furthermore, the existence of a different subgroup of hereditary colon cancer, HNPCC (24,25), suggests that another gene(s) may be involved in the etiology of some colon cancers in addition to the gene on chromosome 5. Evidence for the chromosome localization of an HNPCC gene is based on

positive linkage of the CFS or Lynch syndrome II to the JK gene (26,27). The JK gene has recently been assigned to chromosome 18 because of close linkage of JK to the L2.7 Pst I RFLP at the D18S6 locus (centromeric localization of this linkage group has been suggested; 13).

Karyotypic studies have reported contrasting results on the frequency of chromosome 18 loss in colorectal carcinomas. Muleris et al. recently reported frequent loss of chromosome 18 in sporadic colorectal carcinomas (12 of 18 cases; 10). However, Reichmann et al. analyzed 31 sporadic carcinomas and found that loss of chromosome 18 occurs in less than 20 percent of tumors (8). During the course of this study, two reports also appeared with contrasting results on chromosome 18 allele loss in colorectal carcinomas. Monpezat et al. reported that in 14 patients constitutionally heterozygous at the D18S1 locus on chromosome 18 in normal tissue, 86% had allelic imbalance at this locus when tissue from sporadic colon carcinomas was examined (28). In contrast, a recent study of a Japanese population by Okamoto et al. (22) failed to show any chromosome 18 allele loss at the D18S1 locus in colorectal carcinomas from 7 sporadic and 4 FAP patients constitutionally heterozygous in normal tissue.

Our study shows that at least 44% of colorectal carcinomas lose alleles at the D18S6 locus on chromosome 18. The possibility of a higher prevalence of allelic loss at this locus can not be ruled out because of the possible problem of contaminating normal tissue in tumor samples. The D18S6 locus may have an important localization near the putative CFS or Lynch Syndrome II gene on chromosome 18 because both are closely linked to the JK gene (13,26). The loss of alleles at this locus appears to be specific because our previous studies using this same set of samples to study RFLP loci on chromosomes 2,5,12, and 19 show that loss of alleles in colorectal cancer does not occur randomly (11).

This study is the first report of tumor-specific allele loss on chromosome 18 in colorectal carcinomas from FAP patients. This observation may imply that tumor-specific allele loss occurs on both chromosomes 5 and 18 during hereditary colon carcinogenesis. In two sporadic colorectal carcinomas (patient nos. 18 & 19) we did see allele loss on chromosomes 5 (11) and 18. Based on these molecular data and others (reviewed in ref 9,22), we suggest that colon carcinogenesis involves several specific genetic changes affecting the expression of multiple genes.

Studies showing genetic changes on chromosomes 5 and 18 suggest that more than one tumor- or growth-suppressor gene may be affected in

colorectal cancer. It is possible that several growth suppressor genes on different chromosomes might operate in the regulation of growth in normal colonic epithelium and one or more of these genes may be affected in colorectal cancer. These different genes might code for the specific steps along a metabolic or signal pathway associated with growth suppression, such as transforming growth factor-beta (1). Expression of the differentiated phenotype in non-malignant cells appears to be coupled to growth arrest (29,30). Since several differentiation lineages occur in the colon (6,7), there may be different mechanisms of growth arrest associated with the different cell types. In addition, there may be different growth suppressor mechanisms in the left colon as compared to the right colon because of their different embryologic origins.

Clues as to the role of growth-suppressor genes in normal tissues may come from information on the regulation of cell proliferation in different cell types. For example, cell proliferation of the retinal epithelium occurs only during the first five years of life (2). In contrast, proliferation in the gastrointestinal tract continues throughout life. The normal colorectal epithelium is a constantly renewing cell population with a turnover time of 3-8 days (31). This represents a steady state system whereby cell proliferation is balanced by the loss of an equivalent number of fully differentiated cells. Because DNA synthesis is probably tightly regulated in this highly proliferative organ, more than one growth suppressor mechanism may be functioning to provide a fail-safe growth control system (32). The existence of several growth suppressor mechanisms involving genes on different chromosomes would significantly limit the possibility that a genetically variant cell having uncontrolled DNA synthesis could emerge because several mutational events would be required.

Thus, the etiology of colon cancer may involve inactivation of more than one growth-suppressor gene on different chromosomes. This could explain the concurrent allele losses observed on chromosomes 5 and 18. The hypothesis of the etiology of colon cancer involving several genetic changes on different chromosomes is constant with a clinical impression of extant heterogeneity of this disease.

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