Jpn. J. Cancer Res. 86, 357–360, April 1995

Frequent Genetic Instability in Small Intestinal Carcinomas

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To determine whether genetic instability plays a part in the development of digestive tract carcinomas, we analyzed 3 microsatellite loci isolated from tumors and surrounding normal tissue samples obtained during surgery. The polymerase chain reaction (PCR) technique was used to assess differences between tumor and matched normal DNAs. Replication errors (RERs) were observed in 3 of the 29 cases (10%) of gastric carcinoma and in 11 of the 72 cases (15%) of colorectal carcinoma. None of the 13 (0%) esophageal carcinoma cases showed any RER, but 5 of the 11 cases of small intestinal carcinoma (45%) had RERs, a significantly frequent finding. These results suggest that genetic instability plays an important role in the pathogenesis of small intestinal carcinomas.

Key words: Genetic instability — Hereditary non-polyposis colorectal cancer — Small intestinal carcinoma — Gastric carcinoma — Colorectal carcinoma

Recent evidence has proved that genetic changes to activate dominant oncogenes such as *ras* and inactivate tumor suppressor genes such as APC and p53 are involved in the pathogenesis of digestive tract carcinomas.[1–3] Aaltonen *et al.* showed that colorectal carcinomas were correlated with genetic instability in the microsatellite sequences of hereditary non-polyposis colorectal cancer (HNPCC) family members.[4] The genetic instability of HNPCC-related cancers is often evaluated by using microsatellite markers to detect replication errors (RERs).[5–8] Linkage analysis of HNPCC families has suggested that the genes responsible for the RERs might be located on chromosomes 2p and 3p.[9,10] On each chromosome, hMSH2 and hMLH1, which are DNA mismatch repair genes, were identified and they were shown to be mutated in the carcinomas of patients with genetic instability.[11,12]

Small intestinal carcinomas are very rare, accounting for only about 0.19% of all primary gastrointestinal malignant tumors in Japan.[13] Epidemiological studies have shown that HNPCC family members are diagnosed with cancer of the small intestine at a significantly higher rate than normal (observed/expected ratio is 25).[14] Gastric carcinoma among HNPCC family members was also found at a rate 4 times higher than normally expected. This prompted us to explore whether genetic instability occurs in small intestinal carcinomas. In this study, three microsatellite loci were analyzed by the polymerase chain reaction (PCR) technique. We compared tumor DNAs against matched normal DNAs in sporadic tumors of the small intestine as well as in other digestive tract tumors (esophageal, gastric, and colorectal carcinomas). Genetic instability was significantly more frequent in the cases with small intestinal carcinoma than in those with gastric or colorectal carcinoma. These results indicate that genetic instability is an important consideration in carcinogenesis of the small intestine.

MATERIALS AND METHODS

Tumor samples along with normal tissue were collected from 125 patients with various digestive tract carcinomas diagnosed histologically. Specifically, the tumors included 13 esophageal carcinomas, 29 gastric carcinomas, 11 small intestinal carcinomas, and 72 colorectal carcinomas. All specimens were obtained during surgery, after which 102 paired DNAs from frozen tissues and 23 paired DNAs from paraffin-embedded tissues were prepared as previously described.[15,16]

The method for applying the primers to examine the three microsatellite loci (in parentheses) has been described previously[17–19]: D5S107 (Mfd 27), D17S261 (Mfd 41), and D18S34 (Mfd 26). PCR amplification was performed using a 1×Taq DNA polymerase buffer [10 m*M* Tris (pH 9.0), 50 m*M* KCl, 0.1% Triton X-100], 125 ng of each primer, 1 μg of DNA, 0.6 unit of Taq DNA polymerase, 200 μM of each unlabeled dideoxynucleotide, [^{32}P]dCTP (~3000 Ci/mmol), and 1.5 m*M* $MgCl_2$. All samples were subjected to PCR for 30 cycles of 94°C (1 min), 55°C (2 min), and finally 72°C (2 min). This was followed by 10 min extension at the end. PCR products were denatured at 90°C for 5 min and electrophoretically separated on 6% denaturing polyacrylamide gel for 2 h at 60 W. Gels were then fixed for 15 min in a solution containing 5% acetic acid and 10% methanol, dried and exposed to X-ray film for 24–48 h.

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RESULTS

In an attempt to determine the frequency of genetic instability, we evaluated the frequency of RERs at 3 microsatellite loci in 125 carcinoma samples from the digestive tract (esophagus, stomach, small intestine, and colon), obtained during surgery. PCR-based assays were used to detect differences between the tumor and matched normal DNA banding patterns. All 125 paired samples provided useful data at one or more of the 3 loci as summarized in Table I. RERs were found in 19 of the 125 digestive tract carcinomas (15%), but the frequency varied depending on the part of the digestive tract evaluated. RERs were observed in 3 of the 29 cases (10%) of gastric carcinoma and in 11 of the 72 cases (15%) of colorectal carcinoma. These are relatively low occurrences when compared with the number of RERs found in the small intestinal carcinoma samples. Five out of the 11 (45%) small intestinal carcinoma cases had RERs at 2 or 3 of the loci tested and this frequency was statistically significant (Table I). We examined tumor sites, histology, and the existence of lymph node metastasis in order to better characterize the genetic instability of these 11 small intestinal carcinomas; however, no correlation could be identified (Table II). No RERs were found in any of the 13 esophageal carcinomas tested, confirming that HNPCC tendencies are rare in esophageal carcinoma.

Fig. 1 illustrates some of the size variations found at the 3 loci in the tumor DNAs matched to normal DNAs. Of the 19 tumors exhibiting RERs, 10 (53%) had size alterations at all three microsatellite loci.

DISCUSSION

Epidemiological studies have shown that small intestinal carcinomas possess the components of the HNPCC syndrome more frequently than gastric carcinomas.[14]

Table I. Frequency of RERs Indicating Genetic Instability in Digestive Tract Carcinomas

Part of digestive tract	Microsatellite loci			Total RER(+)[a]	RER(+) at all loci/one or more loci
	D5S107	D17S261	D18S34		
Esophagus	0/13 (0)[b]	0/13 (0)	0/13 (0)	0/13 (0)[c]	0/0 (0)
Stomach	3/29 (10)	3/28 (11)	3/27 (11)	3/29 (10)[d]	3/3 (100)
Small intestine	4/11 (36)	4/11 (36)	4/11 (36)	5/11 (45)[e]	2/5 (40)
Colon	7/61 (11)	7/72 (10)	8/61 (13)	11/72 (15)[f]	5/11 (45)

a) The significance of differences was calculated by using Fisher's exact test by comparison with small intestinal carcinoma.
b) Numbers in parentheses represent percentages.
c, e) $P=0.011$. *d, e*) $P=0.024$. *e, f*) $P=0.032$.

Table II. RER and Clinical Data from Small Intestinal Carcinoma Cases

Case No.	RERs at microsatellite loci			Tumor site[a]	Histology[b]	Lymph node metastasis
	D5S107	D17S261	D18S34			
Case-S1	−	+	+	duo	tub	−
Case-S2	−	−	−	jej	tub	−
Case-S3	−	−	−	duo	tub	+
Case-S4	−	−	−	jej	tub	−
Case-S5	−	−	−	ile	muc	
Case-S6	+	−	+	jej	tub	+
Case-S7	+	+	+	ile	tub	−
Case-S8		−	−	ile	tub	−
Case-S9	+	+	+	jej	tub	−
Case-S10	+	−	+	duo	tub	
Case-S11	−	−	−	duo	AC	−

a) duo, duodenum; jej, jejunum; ile, ileum.
b) tub, tubular adenocarcinoma; muc, mucinous adenocarcinoma; AC, adenocarcinoma with carcinoid features.

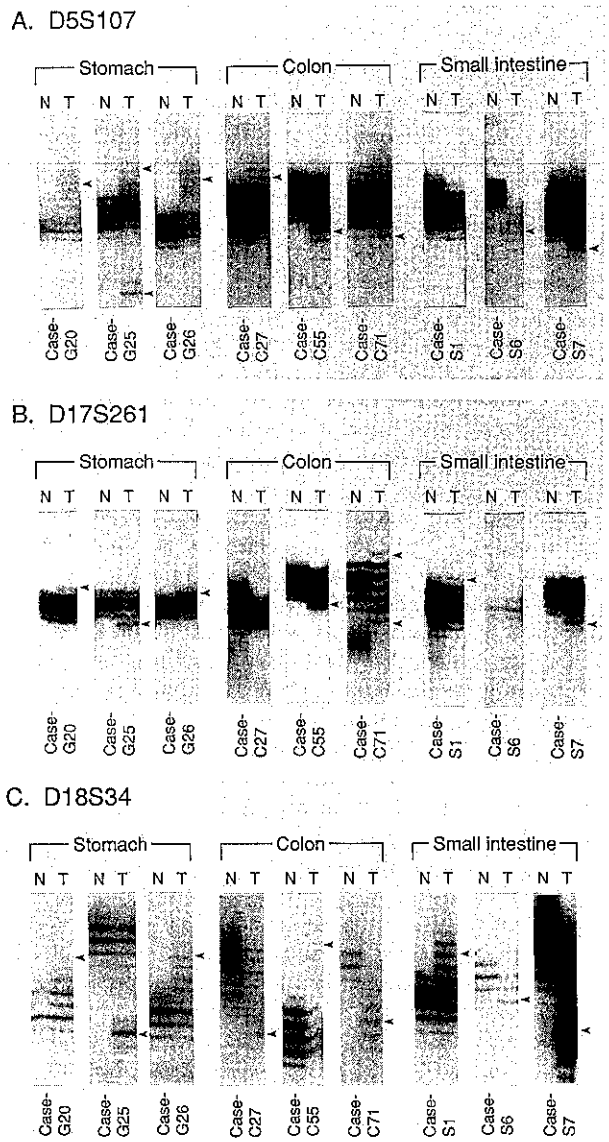

Fig. 1. Genetic instability at 3 microsatellite loci in representative digestive tract carcinoma patients. DNA extracted from the tumor (T) and paired normal (N) tissues of 9 different patients identified by case number (bottom) was amplified using PCR and the products were electrophoretically separated. The deleted or expanded bands (arrows) represent RERs.

We thus wanted to investigate whether small intestinal carcinomas also had more frequent genetic instability. From the digestive tract carcinoma samples obtained during surgery, we found that 10% of the gastric carcinoma cases and 15% of the colorectal carcinoma cases had genetic instability, reflecting the findings of other reports on colorectal carcinomas.[20,21] However the frequency of RERs among gastric carcinomas was lower in our study, possibly due to the different histological distribution of the gastric carcinomas we examined.[5,6] We expected to find less frequent genetic instability among the esophageal carcinoma cases when compared with the gastric and colorectal carcinoma cases, since esophageal carcinoma is a rare component of the HNPCC syndrome. In fact, none of the esophageal carcinomas in our study had any genetic instability, indicating that esophageal carcinogenesis may not be caused by RERs. The most noteworthy result was that 45% of the small intestinal carcinoma cases had genetic instability. Carcinomas of the small intestine involving the duodenum, jejunum, and ileum are very rare, accounting for only about 0.19% of gastrointestinal malignant tumors in Japan.[13] This may be attributable to the rapid transit time as well as the rapid absorption of the intestinal contents, tending to exclude carcinogenic agents from the small intestine. No report to date has addressed the genetic changes occurring in small intestinal carcinomas; this paper may be the first on carcinogenesis of the small intestine. When compared with other digestive tract carcinomas, small intestinal carcinomas show significantly frequent genetic instability, suggesting that genetic instability plays an important role in the pathogenesis of small intestinal carcinoma.

We detected marked genetic instability among small intestinal carcinoma cases in this study of digestive tract carcinomas. However, the frequency of such instability varies depending on the part of the tract involved. For instance, esophageal carcinomas had less frequent genetic instability than gastric or colorectal carcinomas, although small intestinal carcinomas had significantly more frequent occurrence of instability. It is thus clear that the importance of genetic pathogenesis differs in different parts of the digestive tract.

ACKNOWLEDGMENTS

We would like to thank Okazaki Municipal Hospital, Komaki Municipal Hospital, Tajimi Prefectural Hospital, Nagoya National Hospital and Yokoyama Gastrointestinal Hospital for donating the DNA materials. We are grateful to Dr. T. Takahashi of Aichi Cancer Center (Nagoya, Japan) for his advice and encouragement throughout the study. The technical assistance of Ms. E. Uno is also appreciated.

(Received October 24, 1994/Accepted January 18, 1995)

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