

Genetic Instability in Pancreatic Cancer and Poorly Differentiated Type of Gastric Cancer¹

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Abstract

To examine genetic instability during carcinogenesis, we screened 171 carcinomas of the breast, liver, proximal colon, stomach, pancreas, uterine cervix, and ovary for replication error at four microsatellite marker loci on chromosomes 2, 3, and 17. A significantly high incidence of genetic instability was observed in pancreatic (6 of 9 tumors) and gastric cancers (22 of 57 cases). In other types of carcinoma, the incidence of replication error-positive cases was relatively low (3–16%). Among gastric carcinomas, significantly more replication error-positive cases were observed in the poorly differentiated types (16 of 25 cases) than in well differentiated types (3 of 18) ($P = 0.0023$ by Fisher's exact test). Our results suggested that genetic instability is likely to play an important role in development of pancreatic and gastric cancers, particularly poorly differentiated adenocarcinomas.

Introduction

Genetic alterations in simple repeated sequences constitute a newly recognized class of human mutations causing disease (1–3). These regions are genetically unstable and susceptible to RERs³ (1, 4, 5) as judged by their unusually high mutation rates *in vivo* (6) and *in vitro* (7) and by their highly polymorphic nature in the human population. During studies of HNPCC, Aaltonen *et al.* (8) found evidence that genetic instability, probably due to error during replication/repair by strand misalignment, correlated with tumorigenesis in colorectal and other carcinomas that developed in affected members of HNPCC families. Linkage analyses suggested that a variant allele triggering this instability might be located on the short arm of chromosome 2 (9). The concept that multiple genetic alterations affecting protooncogenes and tumor suppressor genes are involved in development of various human cancers is now widely accepted. However, still unclear is whether genetic instability caused by failure of replication fidelity occurs in tumors in a particular organ or in many organs. On the basis of the observations cited above, it would be of great interest to know whether the genetic instability observed in HNPCC-related tumors also occurs in sporadic tumors in other organs. In this study, we compared the sizes of microsatellite loci between tumor DNAs and their corresponding normal DNAs in sporadic tumors of the breast, liver, stomach, proximal colon, pancreas, uterus, and ovary. We found frequent genetic instability in pancreatic carcinomas and in poorly differentiated gastric carcinomas.

Materials and Methods

Tumor DNAs and corresponding normal DNAs were obtained from 171 patients as described previously (10, 18). The tumors included 26 breast

cancers, 29 hepatocellular carcinomas, 18 proximal colon cancers (12 in ascending colon, 6 in caecum), 9 pancreatic cancers, 13 cervical cancers, 19 ovarian cancers, and 57 gastric cancers. Gastric cancers were further classified into three histopathological types according to WHO criteria (11); 18 were well or moderately differentiated adenocarcinomas, 25 were poorly differentiated adenocarcinomas, and 14 were signet-ring cell carcinomas.

The primers used to examine the following four microsatellite loci were described previously: *D2S123* (AFM093xh3) and *D2S136* (AFM172xe7) (12); *D3S1067* (13); and *TP53* (14). The PCR was performed in 25- μ l volumes of a mixture containing 1 \times PCR buffer [6.7 mM Tris (pH 8.8), 16.6 mM NH_4SO_4 , 6.7 μ M EDTA, 10 mM β -mercaptoethanol], 10 pmol each of unlabeled primer and labeled primer with [γ -³²P]ATP (>5,000 Ci/mmol), 20 ng DNA, 0.5 units Taq DNA polymerase, 250 μ M of each dideoxynucleotide, and 5 mM of MgCl_2 . Reactions were heated to 94°C for 5 min and then cycled 35 times; each cycle consisted of 3 min at 55°C, 2 min at 72°C for strand elongation, 2 min at 94°C for denaturation, and 10 min at 72°C for final elongation using a PCR machine (Nippon Genetics, Tokyo, Japan). Following PCR, 5 μ l of reaction product were denatured and then electrophoresed in 6% polyacrylamide gels containing 7.7 M urea and 32% formamide. After electrophoresis, the gels were fixed for 30 min in a solution containing 5% acetic acid and 5% methanol, dried, and exposed to X-ray film for 12–24 h.

Results and Discussion

A total of 171 carcinomas arising in seven different organs (breast, liver, colon, stomach, pancreas, uterine cervix, and ovary) was examined for genetic instability at two (for gastric carcinomas) or four (other carcinomas) microsatellite loci. Fig. 1 shows typical results indicating size alterations at these loci in tumor DNAs in comparison with corresponding normal DNAs. Bands that were not seen in samples amplified from normal DNAs are observed clearly. In some cases (Fig. 1, A, Lanes Pa-2,3, and B, Lanes Ga-47,55), bands observed in normal DNAs have completely disappeared and two bands representing altered molecular weights are seen at the *D2S123* and *D2S136* loci. As we excluded the possibility that DNA samples had been inadvertently switched in these cases by examining other microsatellite loci, it is apparent that RER at these loci occurred either before or at the time clonal growth of a cancer began. At the *TP53* locus, 27 of 166 tumor DNAs examined revealed molecular expansions or contractions (Fig. 1C).

Table 1 summarizes the frequency of RER(+) cases observed at each locus. The overall incidence of RER(+) cases, in which RER for at least one microsatellite locus was detected, was 22% (37 of 171 tumors examined). However, the incidence of RER(+) was strikingly different depending on the organ of origin; RER(+) was significantly more frequent in gastric carcinomas and pancreatic carcinomas than in tumors of the breast, liver, proximal colon, uterine cervix, or ovary. In particular, breast and hepatocellular carcinomas sustained a statistically significantly lower frequency of RER than pancreatic or gastric cancers. Patients in families segregating a mutant allele responsible for HNPCC (2, 8) often develop tumors in the proximal colon, uterus, or ovary (15). Some of these familial tumors have revealed a higher incidence of genetic instability due to replication error during their progression than sporadic tumors (8). Although family histories of the

Received 8/9/93; accepted 9/20/93.

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¹ This work was supported in part by the Ministry of Education, Culture and Science of Japan.

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³ The abbreviations used are: RER, replication error; HNPCC, hereditary non-polytopic colorectal cancer; PCR, polymerase chain reaction; RER(+), RER positive.

patients studied here were not available to us, the tumors revealing RER(+) might reflect inherited susceptibility to genetic instability. An altered replication factor causing instability of the genome could influence to expression of genes which are important for controlling the normal proliferation of cells.

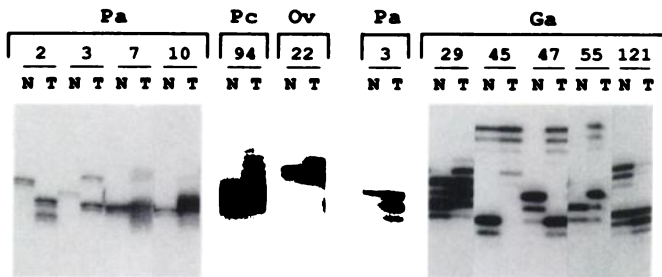
To further characterize genetic instability in the 57 gastric carcinomas, we divided this group of tumors into three histological types (Table 2). It is notable that the frequency of RER was significantly higher in poorly differentiated tumors than in the well differentiated type ($P = 0.0023$ by Fisher's exact test) or the signet-ring cell type ($P = 0.012$). The incidence of RER in poorly differentiated gastric carcinomas was also significantly high in comparison with that in

Table 2 Frequency of genetic instability (RER+) in each histopathological type of gastric carcinomas^a

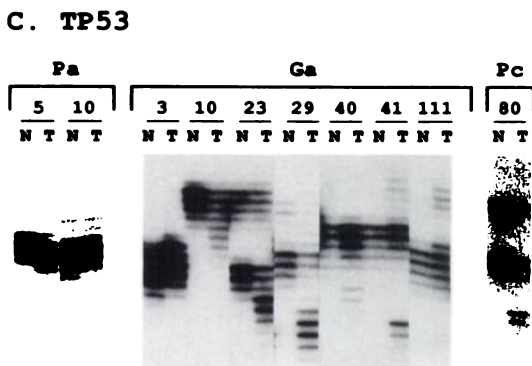
Histological type	Frequency with alteration		Total RER (+)
	D2S136	TP53	
I Well differentiated	2/18 (11)	2/18 (11)	3/18 (17)
II Poorly differentiated	10/25 (40)	13/22 (59)	16/25 (64)
III Signet-ring cell	1/14 (7)	2/14 (14)	3/14 (21)
Total	13/57 (23)	17/54 (31)	22/57 (39)

^a P value for the difference between frequencies of RER (+) in type I versus type II was 0.0023, and that for type II versus type III was 0.012, calculated by Fisher's exact test. Numbers in parentheses, percentages.

A. D2S123



B. D2S136



C. TP53

Fig. 1. Analysis of genetic instability in paired normal (N) and tumor (T) DNA at loci D2S123 (A), D2S136 (B), and TP53 (C). The sizes of PCR products were around 100 (B and C) or 200-base pair (A). In tumor DNAs, abnormal patterns indicating expansion or deletion are shown at each microsatellite locus. Normal alleles appear as major bands with their ladders. All patterns were reproducible in each repeated assay. Patients numbers are shown above the lanes. Pa, pancreatic cancer; Ga, gastric cancer; Pc, proximal colon cancer; Ov, ovarian cancer.

Table 1 Frequency of genetic instability (RER+) in various cancers by site

Cancer site	No. of tumors with alteration			Total RER(+) ^a
	D2S123/D2S136	D3S1067	TP53	
Breast	0/26 (0) ^b	0/26 (0)	1/26 (4)	1/26 (4) ^c
Liver	0/29 (0)	0/29 (0)	1/29 (3)	1/29 (3) ^d
Proximal colon	1/18 (6)	1/18 (6)	2/18 (11)	2/18 (11) ^e
Stomach	13/57 (23)	NC	17/54 (31)	22/57 (39) ^f
Pancreas	4/8 (50)	2/8 (25)	4/9 (44)	6/9 (67) ^g
Uterus	0/12 (0)	1/13 (8)	1/11 (9)	2/13 (15) ^h
Ovary	1/19 (5)	2/18 (11)	1/19 (5)	3/19 (16) ⁱ
Total	19/169 (11)	6/112 (5)	27/166 (16)	37/171 (22)

^a Significant differences were calculated by the Fisher's exact test by comparisons with gastric cancer or pancreatic cancer.

^b Numbers in parentheses, percentage; NC, not examined in this locus.

^{c-f} $P = 0.0005$.

^{d-f} $P = 0.0002$.

^{e-f} $P = 0.025$.

^{c-g} $P = 0.0003$.

^{d-g} $P = 0.0002$.

^{e-g} $P = 0.0061$.

^{g-h} $P = 0.022$.

^{g-i} $P = 0.013$.

breast ($P = 0.000004$), liver ($P = 0.000001$), proximal colon ($P = 0.0006$), uterine cervix ($P = 0.005$), or ovary ($P = 0.002$).

Among the 25 poorly differentiated adenocarcinomas of the stomach which were examined at the only two loci, eight were RER(+) at both the loci. Three of the 9 pancreatic carcinomas were also RER(+) at the 2 or more loci. These results support the genomic instability in these type of tumors.

Our data clearly imply that genetic instability plays an important role in development of pancreatic cancer and of gastric cancer, particularly in the poorly differentiated type. Genetic alterations previously reported in gastric carcinomas have included amplifications of the *erbB-2* and *K-sam* genes and point/frame-shift mutations of the *K-ras*, *p53*, and *APC* genes; however, the genetic alterations are observed relatively infrequently except in the case of *APC* (16, 18). Among these mentioned, only amplification of the *K-sam* gene seems to be specific to the poorly differentiated type of gastric cancer (17).

The microsatellite instability reported here is the most frequent genetic alteration thus far detected in poorly differentiated gastric carcinomas. It appears to be specific to this type of gastric cancer; in contrast, somatic mutations of the *APC* gene are frequent in well differentiated gastric carcinomas but very rare in the poorly differentiated type (18). Previously, we had examined gastric cancers for somatic mutations in part of the *APC* gene, which is responsible for familial adenomatous polyposis and appears to be one of the tumor suppressor genes associated with development of sporadic colorectal carcinomas (19). In those studies, we found somatic mutations in 20–40% of well differentiated gastric cancers but detected none in 19 poorly differentiated tumors. The data presented here further support our previous hypothesis that pathologically distinct subtypes of gastric carcinomas undergo different genetic pathways during tumorigenesis. Furthermore, generally earlier onset of poorly differentiated gastric cancers (55 years old) compared to well differentiated types (65 years old) may imply the presence of a genetic factor associated with susceptibility to genetic instability in patients who develop the poorly differentiated type of gastric carcinoma.

We have shown that the genetic instability of loci containing dinucleotide repeats is magnified in some pancreatic cancers and gastric cancers of poorly differentiated type. If the mechanism for this instability and the stage at which it occurs during development/progression of carcinogenesis in these cancers could be defined more clearly, a test for alteration in microsatellite loci might provide useful prognostic information and contribute to an understanding of the biological significance of such myotonus.

Acknowledgments

We gratefully acknowledge gifts of DNA materials from Drs. Akira Horii, Mitsuru Emi, Hiroki Nagase, Shigetoshi Ichii, Hiroko Saito, and Yoshiyuki Fujiwara and the technical assistance of Kiyoshi Noguchi at the Cancer Institute, Tokyo, Japan.

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Cancer Res 1993;53:5087-5089.

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