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Original Paper

Microsatellite Instability in Gastric Carcinoma with Special References to Histopathology and Cancer Stages

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To study the molecular mechanism of gastric carcinogenesis, the frequencies of microsatellite instability were evaluated with seven dinucleotide repeat loci in 59 patients with gastric carcinoma. Microsatellite instability at two or more loci was found in 41.5% (17/41) of advanced gastric carcinoma, 21.4% (3/14) of early gastric carcinoma, but not in remnant gastric carcinoma (0/4), with an overall frequency of 33.9% (20/59). Diffuse gastric carcinoma had a similar prevalence (32.1%, 9/28) to intestinal gastric carcinoma (40.7%, 11/27). The frequency of microsatellite instability in gastric carcinoma was not significantly different with respect to age, sex and *Helicobacter pylori* infection. Microsatellite instability tended to occur more frequently in cancers of the cardia (62.5%, 5/8) compared with cancers of other stomach regions (31.9%, 15/47), but the difference was not statistically significant. These data suggest that microsatellite instability occurs in early gastric carcinoma and its occurrence increases during tumour progression. Furthermore, its frequency was independent of age, gender, histological types and *Helicobacter pylori* infection.

Key words: microsatellite instability, replication error, gastric carcinoma, histopathology, *Helicobacter pylori*

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INTRODUCTION

GASTRIC CARCINOMA has marked heterogeneity in morphology and function [1]. Two distinctive histopathological entities, the intestinal and diffuse type, have been shown to differ drastically in the phenotypic characteristics of the precursor stage [2, 3]. Environmental factors play an important role in gastric carcinogenesis [4]. However, molecular mechanisms of cancer promotion by environmental influences, such as diet and *Helicobacter pylori* (*H. pylori*) infection, await further elucidation [1–4]. Furthermore, existing data concerning genotypic abnormalities of gastric carcinoma including oncogenes, tumour suppressor genes, and growth factors remain inconclusive [2].

The polymorphic nature of microsatellites, together with their abundance and even genomic distribution, are responsible for their emergence as ubiquitous genetic markers [5]. Microsatellite instability, manifesting as expansion or contraction of DNA within repeated sequences, has been documented as a new landmark of colorectal carcinoma and other tumours [5–9]. Abnormalities of the mismatch repair gene on chromosomes 2 and 3 may be responsible for replication error (RER) [10–14]. RER+ colonic carcinomas are significantly associated with distinctive clinicopathological characteristics [15–17]. Similar phenomena of RER have recently been reported in gastric carcinomas [18–22]. Nevertheless, the frequency of RER+ phenotypes varied from 22.7 to 62.5% in different studies, and the relationship between microsatellite instability and clinicopathological characteristics of gastric carcinoma remains controversial. Moreover, previous studies on microsatellite instability of gastric carcinoma have been performed predominantly in advanced stage cancers. It is, thus, unclear whether a similar frequency of RER also occurs during the early phase of gastric carcinogenesis [18–22]. In this study, we determined the frequency of RER in 59 patients with gastric carcinoma with special reference to histopathological variables and cancer stage.

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PATIENTS AND METHODS

Patients and DNA extraction

Paired gastric tissues from tumoral and non-tumoral sites were obtained from 59 patients with gastric adenocarcinoma during surgery from January 1992 until June 1993. The tissues

were immediately frozen in O.C.T. embedding compound (Miles Scientific, U.S.A.) and stored at −80°C until used. All tissues of gastric carcinoma were verified histologically by the same pathologist (C.-T. Shun). Based on the criteria of the Japanese Gastric Cancer Study Group [23], 14 patients were classified as early gastric carcinoma (EGC), 41 patients as advanced gastric carcinoma (AGC), and 4 patients as remnant gastric carcinoma (RGC) which occurred after surgery for peptic ulcer. Cryostat sections were prepared of these tissues [24], and only samples containing predominantly neoplastic cells were used to prepare genomic DNA. DNA was extracted by the conventional procedure. From the same patient, tissue from the non-tumoral area of the stomach, showing no dysplasia or metaplasia, was also dissected, and DNA was similarly extracted to serve as a control. Relevant demographic and clinicopathological information for each patient was obtained from medical records, and none of the patients in this study belonged to cancer families.

The status of *H. pylori* infection was determined by serological examiniation of IgG antibodies against *H. pylori* by using an enzyme-linked immunosorbent assay (Amrad, Australia) as well as histological examination.

Microsatellite analysis

Seven primers, including *D2S114* (chromosome 2p16-21), *D2S123* (chromosome 2q21-22),*D2S123* (chromosome 3p21-23), *D5S395* (chromosome 5p13-14), *D10S193* (chromosome 10p11-12), *D10S197* (chromosome 10q11-12), *D17S785* (chromosome 17q23-25), for the analysis of microsatellite instability were obtained from Research Genetics, U.S.A. Polymerase chain reaction (PCR) was performed in 25 μl reaction volumes containing 5 μM of each primer, 0.125 mM dATP, 1.25 mM each of dGTP, dCTP and dTTP, 3 μ Ci [α-^{35}S] dATP, 25 ng of the patient's DNA, and 0.75 units of Taq DNA polymerase (Boehringer Mannheim, Germany). Reaction conditions were 30 s at 94°C, 75 s at 55°C, and 15 s at 72°C for 27 cycles, followed by a final extension of 5 min at 72°C. The PCR products were diluted in a ratio of 3:2 by loading buffer, heated at 95°C for 5 min and loaded (5 μl) on to 7% polyacrylamide sequencing gels. After electrophoresis, gels were dried at 80°C and exposed to X-ray film for 24–72 h. The band patterns between the tumoral and non-tumoral tissues for each patient were compared contemporaneously. To avoid an artifact produced by PCR, all tests were duplicated, and the RER (+) tumours were defined by the presence of microsatellite alterations in at least two different loci.

Statistical analysis

Odds ratios with 95% confidence intervals were calculated with the exact method for the prevalence of RER in age, sex, *H. pylori* infection, histology, cancer stage and tumour location. A P value less than 0.05 was considered statistically significant.

RESULTS

Among the 59 patients examined, 20 (33.9%) had microsatellite instability at two or more chromosomal loci (Table 1). Instability was found in 17 of 41 (41.5%) patients with AGC, in 3 of 14 (21.4%) with EGC, and in none of 4 patients with RGC. The RER+ tumours showed patterns of contraction or expansion of the microsatellite repeats in their DNAs as compared to that of corresponding non-tumoral DNAs. Typical examples are shown in Figure 1, and all patterns were reproducible with duplicated assays. In addition, cases 10 and 45 also showed loss of heterozygosity at the *D5S395* marker.

The relationship of RER+ phenotype and clinicopathological variables is summarised in Table 2. Age, gender, *H. pylori* infection and histological types were not associated with the RER+ phenotype. Cancer stage tended to affect the frequency of RER (odds ratio: 2.56, 95% confidence interval: 0.56–16.42), but the frequency of RER in EGC (21.4%, 3/14) was not

Table 1. Distribution of microsatellite allelic shifts among RER (+) tumours

Case no.	*D2S114*	*D2S123*	*D3S1260*	*D5S395*	*D10S193*	*D10S197*	*D17S785*
2	−	+	−	−	+	−	−
10	−	+	+	LOH	−	−	+
12	−	−	+	−	−	+	−
13	−	−	+	+	−	+	−
14	−	−	+	+	+	+	+
18	+	+	−	−	−	−	+
26*	−	+	+	−	−	−	−
27	+	+	−	−	−	+	−
31	−	+	+	+	−	−	+
32	+	+	+	+	−	+	−
33	+	−	−	+	+	−	+
34	−	+	−	−	−	+	−
35	+	+	−	−	+	−	−
37	+	+	+	+	−	−	+
38	+	+	+	+	+	−	+
43	−	+	−	−	+	−	−
45	+	+	+	LOH	−	+	+
46*	−	+	+	−	−	−	−
51	−	+	−	−	+	−	−
56*	+	+	−	−	−	−	+

(+) indicates detected shifts by microsatellite assays. RER, replication error; LOH, loss of heterozygosity.
*Cases 26, 46 and 56 were early gastric cancer, while the others were advanced gastric cancer.

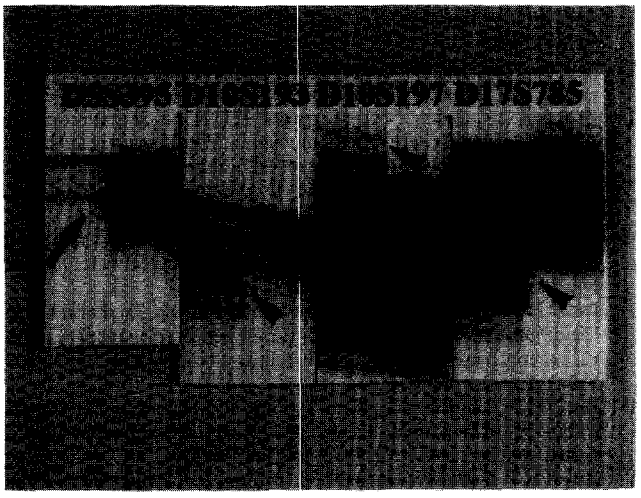

Figure 1. Autoradiographs of microsatellite markers assays. The corresponding loci are indicated above each panel. Paired sample lanes with tumour (right) and normal (left) are aligned on each locus. Arrowheads show shifts with smaller alleles or larger alleles at different loci. Two cases also show loss of heterozygosity at locus *D5S395* (arrow).

Table 2. Microsatellite instability and clinicopathological variables in gastric carcinoma

Variable*		RER+†(%)		Odds ratios (95%CI)‡
Age				
	≤ 60	6/19	(31.6)	1.00
	61–70	7/20	(35.0)	1.08 (0.24–4.87)
	> 70	7/16	(43.8)	1.11 (0.23–5.35)
Sex				
	Male	13/33	(39.4)	1.00 (0.29–3.61)
	Female	7/22	(31.8)	
H. pylori§				
	Positive	12/34	(35.3)	0.86 (0.23–3.35)
	Negative	7/18	(38.9)	
Histology				
	Diffuse	9/28	(32.1)	0.69 (0.20–2.38)
	Intestinal	11/27	(40.7)	
Stage				
	Advanced	17/41	(41.5)	2.56 (0.56–16.42)
	Early	3/14	(21.4)	
Location				
	Cardia	5/8	(62.5)	3.47 (0.59–25.33)
	Non-cardia	15/47	(31.9)	

*Excluding four remnant cancers which showed negative results for RER; †Number with positive replication error (RER+)/number examined; ‡CI, confidence interval; §Determined by IgG antibody to *H. pylori* as well as by histology. 3 patients who did not have stored sera for testing were excluded.

statistically different from that in AGC (41.5%, 17/41) ($P=0.18$). Moreover, RER seemed to be more common in cancers of the cardia (62.5%, 5/8) than cancers of other areas of the stomach (31.9%, 15/47), but this difference was not statistically significant (odds ratio: 3.47, 95% confidence interval: 0.59–25.33, $P=0.12$).

DISCUSSION

Microsatellite instability due to RER is a novel genetic mechanism in tumorigenesis [5]. The prevalence of the RER+ phenotype varies with primary tumour sites, and a high rate of RER+ phenotype has been demonstrated in colorectal, gastric, pancreatic, endometrial and pulmonary carcinomas [5–9]. The reported frequency in gastric carcinoma ranges from 22.7 to 62.5% [18–22], and the results of the current study (33.9%) fall within this range. Since a certain proportion of stromal cells interposing with neoplastic cells in tumoral tissues may affect the frequency of RER, it is difficult to compare the results of different studies. However, our data support the fact that RER occurs at relatively high frequency in sporadic gastric carcinoma.

In colonic carcinoma, RER predominates in poorly differentiated tumours [15, 16]. Nevertheless, the association of pathological type and RER in gastric carcinoma remains controversial [18–22]. Earlier reports showed that it occurs predominantly in poorly differentiated carcinoma [18], while later reports did not reveal any difference with respect to histological type [19, 21]. In this study, we adopted Lauren classification, which has been shown to adequately reflect the epidemiological and molecular features of gastric carcinoma [2]. Our results showed that the frequency of RER in diffuse (32.1%) and intestinal (40.7%) gastric carcinoma were not statistically different. This implies that RER may have a common pathogenic role for both histological types, or that RER may be the common manifestation of two different pathogenic mechanisms.

Another important question is when RER occurs in gastric carcinogenesis. Although RER has been shown to occur in early colorectal carcinogenesis in both *in vitro* and *in vivo* studies [25], most data available for RER in gastric carcinoma originate only from advanced stage tumours [18–20]. Rhyu and associates argued that RER was a late event in gastric carcinogenesis because RER rarely occurred in precancerous dysplastic tissues of their study [19]. Recently, RER was also found in early gastric carcinoma and thought to play a role in tumour progression [21]. In our series, 17 of 41 (41.5%) patients with AGC, and 3 of 14 (21.4%) with EGC were found to have microsatellite instability. Our study also indicates that RER may occur in early stage gastric carcinoma, and the frequency increases as the tumour progresses.

RER has been shown to occur more frequently in cancer of the proximal colon than other parts of the colon [15, 16], but the influence of tumour location on RER has not been discussed in previous studies of gastric carcinoma [18–22]. In our study, RER appeared to be more common in cancers of the cardia than those of other areas, although the numbers were small. Such a tendency is interesting because some epidemiological evidence suggests that the incidence of cancers of cardia has increased rapidly in recent years, and cardia cancers may have biological features distinguishable from cancers of other areas of the stomach [26]. However, further studies are needed to confirm this.

H. pylori infection has recently been reported as an important risk factor for gastric carcinogenesis, while remnant stomach after peptic ulcer surgery is regarded as a paradigm of multistep

neoplastic process [27, 28]. However, studies of the relationship between molecular carcinogenesis of the gastric remnant and *H. pylori* infection remain sparse [1–4]. In the present study, no obvious association was found between RER+ phenotype and clinicopathological variables such as age, sex and *H. pylori* infection. Moreover, none of the four cases of remnant carcinoma examined in this study showed RER. However, because of the limited numbers, further study is needed to elucidate whether different pathways of tumorigenesis exist for this special type of gastric cancer.

In conclusion, our results suggest microsatellite instability occurs in early gastric carcinoma, and its frequency increased during tumour progression. The frequency of microsatellite instability is not significantly different with respect to age, gender, histology and *H. pylori* infection. Future studies of mismatch repair gene mutations, such as *hMSH2* and *hMLH1*, and of the relationship to other tumour suppressor genes may help to elucidate the molecular carcinogenesis of gastric carcinoma.

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