Microsatellite Instability in the Progression of Gastric Carcinoma

Ja-Mun Chong,1 Masashi Fukayama,2 Yukiko Hayashi, Touichirou Takizawa, Morio Koike, Motoko Konishi, Rei Kikuchi-Yanoshiba, and Michiko Miyaki

Department of Pathology, Tokyo Metropolitan Komagome Hospital, 3-18-22 Honkomagome [J.-M. C., M. F., Y. H., T. T., M. K.], and Department of Biochemistry, Tokyo Metropolitan Institute of Medical Science, [M. K., R.-K. Y., M. M.], Bunkyo-ku, Tokyo 113, Japan

Abstract

Somatic alteration of numerous microsatellite sequences, caused by DNA RER, has been reported in hereditary nonpolyposis colorectal cancer and certain sporadic cancers of the proximal colon (1–3). This type of genetic alteration is different from that in familial polyposis coli and sporadic cancers of the distal colon. In addition to the mutation in the APC gene, most loci encoding tumor suppressor genes undergo somatic losses during tumorogenesis in the latter group of cancers (4–6). On the other hand, there is no correlation between microsatellite instability and chromosomal losses, such as 5q or 17p, in colorectal carcinomas (2).

Gastric carcinoma is one of the most common malignancies in the world, especially in Japan and South America. Although the molecular basis of the development of gastric carcinoma remains unclear, there have been many attempts to apply the same analysis which has been effective in colon cancers (7, 8). Some tumor suppressor genes are similarly deleted in the intestinal type of gastric carcinoma as early as the intramuscosal stage (8). However, very little information is available regarding microsatellite instability in gastric carcinomas, particularly in terms of the pathological features of the carcinomas and the familial tendency to gastric carcinoma or EBV, which has been determined to be associated with some gastric carcinomas (9). In the present study, we also evaluated the correlation between instability and somatic losses of chromosome 5q and 17p to find a possible sequence of genetic alterations which might be different from that in colon carcinoma.

Materials and Methods

Patients and Samples. A total of 76 pairs of primary gastric carcinoma tissue and corresponding normal tissue were obtained from 75 patients at the Tokyo Metropolitan Komagome Hospital from 1988 to 1993. The patient group consisted of 51 men and 24 women (ages 34 to 89 years; mean, 64.5). The samples were taken immediately after resection and frozen in dry ice-hexane for DNA analysis. The remaining tissue was routinely processed for histopathological analysis. The frozen samples were separated into two parts; one-half was used for DNA-extraction, and the other one-half was subjected to cryostat sectioning to confirm the amount of carcinoma cells in the tissue. In all of the samples, at least 30% of the total cells were carcinoma cells.

Gastric carcinomas were classified into three histopathological types: intestinal type (well-to moderately differentiated type), solid type, and signet-ring/schwann type. The depth of a gastric carcinoma is an important factor in analyzing its progression. The invasive depth was determined histologically as either early (limited to the submucosa or muscularis propria) or advanced (beyond the muscularis propria). The familial histories of the patients were obtained by reference to their clinical charts.

DNA Extraction. DNA was extracted by a phenol-chloroform procedure as reported previously (4).

Analysis of LOH. LOH was examined on two chromosomal loci, 5q and 17p, using PCR-based RFLP (10). Genomic DNA (500 ng) was used as the template in a total reaction volume of 50 ¡¿l containing 500 ¡¿M of each primer, 200 ¡¿M of each deoxynucleotide triphosphate, 1X PCR buffer, and 2.5 units of Taq DNA polymerase (Perkin-Elmer Cetus, Norwalk, CT).

For the analysis of LOH in the p53 gene on chromosome 17p, primer pairs were chosen which flanked a BstUI RFLP site within exon 4 and anMspI RFLP site within intron 6, respectively: 5'-GATGCTGTCCGCGGAC-GATATT-3' and 5'-CTGCTGCAAGTCACAGACTGCC-3' for p53 exon 4; and 5'-AGTCTGTGTTCGCACTGG-3' and 5'-AGGTCAATATAGCAGG-3' for p53 intron 6. To analyze LOH on 5q, primers were chosen which flanked an Rsal RFLP site within exon 11 of the APC gene (5'-GGACGATCAGGCCCATTGCAAA-3' and 5'-GGCTACATCTCCAAAAGT-CAA-3') and which flanked a variable insertion polymorphism within exon 10 of MCC (5'-TACGATTTCAATGCCCAACAA-3' and 5'-CTGAAGTATGCTT-CAAAC-3').

Thirty cycles of PCR were programmed as 1 min at 94°C, 1 min at 58°C, and 1 min at 72°C. After amplification, 5 ¡¿l of PCR products were digested with 10—50 units of appropriate restriction enzymes, except for PCR products of the MCC gene. For the MCC gene, restriction enzyme digestion was not necessary, because the polymorphism type is a variable number of tandem repeats. The digests and PCR products of the MCC gene were then electrophoresed on polyacrylamide gels, which were stained with ethidium bromide and photographed under UV light.

Microsatellite Instability. Seventy-six pairs of samples were examined for microsatellite instability. The primers were targeted to examine two microsatellite loci on chromosomes 2 (D2S123) and 6 (MFDR4; Refs. 11 and 12). The reaction mixture (6 ¡¿l) contained 200 ng of genomic DNA, the proper pair of each primer (0.2 ¡¿M), 25 ¡¿M of each deoxynucleotide triphosphate, 1X PCR buffer, Taq polymerase (Perkin-Elmer Cetus, Norwalk, CT), and [α-32P]dCTP. After heating at 94°C for 5 min, PCR was performed for 35 cycles of 1 min at 55°C, 1 min at 72°C for strand elongation, and 1 min at 94°C for denaturing. Final elongation was performed for 10 min at 72°C. Reaction product (5 ¡¿l) was then denatured and electrophoresed in 6% polyacrylamide gel containing 7 M urea. After electrophoresis, the gel was fixed on paper and exposed to X-ray film for 48—72 h.

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1 To whom requests for reprints should be addressed.
2 Present address: Department of Diagnostic Pathology, Kanto Teishin Hospital, Tokyo, Japan.
3 The abbreviations used are: RER, replication errors; RER (+), RER positive; EBV, Epstein-Barr virus; LOH, loss of heterozygosity; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; RER(-), RER negative.
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Fig. 1. LOH on chromosomal loci 5q and 17p in gastric cancer. DNA band sizes are indicated in base pairs (bp). T, tumor; N, normal tissue. LOH affecting exon 4 of p53 (A), intron 6 of p53 (B), exon 11 of APC (C), and exon 10 of MCC (D).

Virological Studies. EBV was examined in the 76 cases in which LOH and microsatellite instability were analyzed. As reported previously (13), EBV genome was detected by in situ hybridization using EBV-encoded small RNA-1 (EBER-1) transcripts.

Statistical Analysis. A correlation analysis of microsatellite instability, LOH, EBV, and pathological factors was evaluated by Fisher’s exact probability test.

Results

Genetic Alteration in Gastric Carcinoma. Among the 76 cases examined by PCR RFLP, LOH was found in 44 cases on chromosome 5q (58%; 34% at APC locus and 41% at MCC locus) and in 42 cases on 17p (55%; 45% at p53 exon 4 and 23% at p53 intron 6). LOH on 5q was observed in 13 of 44 informative cases (30%), and LOH on 17p was found in 17 of 42 informative cases (40%; Fig. 1; Table 1).

Microsatellite instability (RER-positive phenotype) was evident when the tumor DNAs gained new bands compared to their normal counterparts (Fig. 2). Deletion of the bands (LOH of microsatellite allele) was rarely observed, but it was not considered to be a microsatellite alteration in the present study. RER (+) was observed in 17 of 75 cases (23%) at D2S123 and in 15 of 75 cases (20%) at MFD47. Overall, RER (+) was found in 25 of 76 cases (33%).

Genetic Alteration and Pathological Features of Gastric Carcinoma (Tables 1 and 2). Frequencies of RER(+) and 5q LOH were similar in intestinal-type (40 and 32%) and solid-type (47 and 54%) gastric carcinoma but were rare in signet-ring/scirrhous-type (9 and 0%). However, the incidence of 17p LOH in the signet-ring/scirrhous-type (29%) was similar to that in the solid-type (29%). This fact indicated that the rarity of RER(+) and 5q LOH in the signet-ring/scirrhous-type was not due to the contamination of normal tissue in tumor DNAs.

As for the invasive depth of the carcinoma, RER (+) was found much more frequently in the advanced stage (22/56) than in the early stage (3/20). Similar findings were seen with 17p LOH, the incidence of which was 15 of 31 in advanced and 2 of 11 in early carcinoma. On the other hand, the incidence of 5q LOH did not significantly increase in the advanced stage (11/32), relative to early carcinoma (3/12).

RER (+) occurred in combination with other genetic abnormalities. The incidence of 5q LOH and 17p LOH was significantly higher in RER(+) carcinoma than in RER (−) carcinoma (Table 2). There were no EBV-positive cases among the 25 RER(+) cases. However, 8 EBV-positive cases (including 3 cases of early carcinoma, one of which is an intramucosal carcinoma) were observed among the 51 RER(−) cases. This difference was statistically significant (P < 0.05). Furthermore, LOH on 5q or 17p was also rare in EBV-positive cases (1 of 6 and 0 of 5, respectively), whereas LOH was seen in 13 of 33 and 17 of 35 of EBV-negative cases, respectively.

Five of 25 RER(+) cases (20%) and 10 of 51 RER(−) cases (20%) had family members from the first to third degree who contracted gastric carcinoma. There were 7 RER(+) cases (28%) and 16 RER(−) cases (28%) in which family members from the first to third degree contracted carcinomas of other organs including the esophagus, colon, breast, lung, uterus, and urinary tract. No correlation was found between familial history and microsatellite instability (Table 2).

There were 7 multiple-primary cases, e.g., double gastric carcinoma or gastric carcinoma complicated by colon carcinoma (29%), among the 24 RER(+) cases, and 17 multiple-primary cases among the 51 RER(−) cases (33%). We found no correlation between the incidence of multiple-primary cases and genetic instability.

Discussion

Microsatellite instability was demonstrated in 25 of 76 gastric carcinomas. This incidence is comparable with those in other reports, although these other reports were on a smaller scale than the present study (14–16). When genetic alteration is correlated with the development of gastric carcinoma, the histological type of the carcinoma is one of the most important factors to be taken into account. In the present study, the RER(+) phenotype was observed more frequently in the intestinal and solid types of carcinoma, compared to signet-

![D2S123 Mfd 47](image)

**Fig. 2.** Microsatellite instability in paired tumor (T) and normal tissue (N) DNA at loci D2S123 on chromosome 2 and MFD47 on chromosome 6. Examples of alterations include a loss of repeat (patient 38) and the gain of new shorter (patient 1) or longer (patient 56) repeats.

<table>
<thead>
<tr>
<th>Histological type</th>
<th>Frequency of allele loss</th>
<th>Frequency of alteration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5q</td>
<td>17p</td>
</tr>
<tr>
<td>Intestinal</td>
<td>6/19 (32)</td>
<td>11/21 (52)</td>
</tr>
<tr>
<td>Solid</td>
<td>7/13 (54)</td>
<td>2/7 (29)</td>
</tr>
<tr>
<td>Signet-ring /scirrhous</td>
<td>0/12 (0)</td>
<td>4/14 (29)</td>
</tr>
<tr>
<td>Total</td>
<td>13/44 (30)</td>
<td>17/42 (40)</td>
</tr>
</tbody>
</table>

Table 1 Frequency of LOH and microsatellite alteration in each histological type of gastric carcinoma.
Table 2 Frequency of each stage of depth of invasion, LOH at 5q and 17p, EBV, family history, and multiple-primary carcinoma in the RER(+) cases and the RER(-) cases

<table>
<thead>
<tr>
<th></th>
<th>Early/advanced</th>
<th>5q LOH</th>
<th>17p LOH</th>
<th>EBV*</th>
<th>Family history</th>
<th>Multiple-primary*</th>
</tr>
</thead>
<tbody>
<tr>
<td>RER(+)</td>
<td>25</td>
<td>3/22f</td>
<td>9/15f</td>
<td>8/11f</td>
<td>0/25f</td>
<td>12/25</td>
</tr>
<tr>
<td>RER(-)</td>
<td>51</td>
<td>17/34f</td>
<td>5/20f</td>
<td>9/30f</td>
<td>8/51</td>
<td>26/51</td>
</tr>
</tbody>
</table>

* Number of cases in the early stage versus number of cases in the advanced stage of gastric carcinoma in the RER(+) cases and the RER(-) cases.

** Significantly different (P < 0.05) by Fisher’s exact test.

*** Significantly different (P < 0.01).

ring/scirrhous carcinoma. This finding is compatible with a previous observation based on morphology that the developmental pathway of the latter type is different from that of the others (17).

Microsatellite instability is a late event in the development of gastric carcinoma; the RER(+) phenotype occurred much more frequently in the advanced stage than in the early stage. This instability was frequently accompanied by 5q or 17p LOH. It has been well-established that the biological behavior of advanced gastric carcinoma differs from that of early carcinoma. Lymph node and hematogenous metastasis are extremely rare in the early stage but become much more common in the advanced stage. Although recent studies have suggested that changes in several mismatch repair genes may be related to the RER(+) phenotype in hereditary nonpolyposis colorectal cancer (18, 19), the precise biological implications of the RER(+) phenotype in gastric carcinomas are not known. However, our present results suggest that the RER(+) phenotype represents an important genetic alteration associated with tumor progression from the early to the advanced stage of gastric carcinoma.

Aaltonen et al. (2) reported that there was no correlation between the RER(+) phenotype and chromosomal losses in colorectal carcinoma (20). On the other hand, both genetic changes, i.e., 5q and 17p, were observed simultaneously in gastric carcinomas, which suggests that the genetic sequence may be different, even in gastric carcinoma showing similar chromosomal losses.

There is little information available regarding the etiological agents of, or genetic liability to, gastric carcinoma. Recently, we reported that EBV-encoded small RNA (EBER-1) was identified by in situ hybridization in nearly all of the tumor cells in 8 of 72 cases of gastric carcinomas, which suggests that EBV might be a factor initiating EBV-associated gastric carcinoma. In the present study, we have shown that microsatellite instability in pancreatic cancer and poorly differentiated type of gastric cancer. Cancer Res., 53: 1044-1049, 1993.

In the present study, we have shown that microsatellite instability in gastric carcinoma is a late genetic event and is accompanied by LOH of tumor suppressor genes, although specific subtypes, such as the signet-ring/scirrhous type of carcinoma or EBV-associated carcinomas, might develop along a different genetic pathway. Additional studies are required to determine whether the RER(+) phenotype and chromosomal losses have any prognostic significance in advanced gastric carcinomas, as is found in colon carcinomas (20).

Acknowledgments

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