Original article

Microsatellite instability in patients with gastric remnant cancer

ATSUSHI NAKACHI, HIROSHI MIYAZATO, HIDEAKI SHIMOJI, SHUNGO HIROYASU, TSUTOMU ISA, MASAYUKI SHIRAISHI, and YOSHIHIRO MUTO

First Department of Surgery, Ryukyu University School of Medicine, Okinawa 903-0215, Japan

Abstract:

Background. About 2% of patients who undergo partial distal gastrectomy for gastroduodenal diseases develop gastric remnant cancer 10 to 30 years after the gastrectomy. It is important in clinical practice to determine a molecular marker to identify patients susceptible to gastric remnant cancer.

Methods. We investigated nine gastric remnant cancers (from nine individuals who had gastrectomies for primary gastric cancer or gastroduodenal ulcer) for microsatellite instability (MSI) at six loci, using the polymerase chain reaction (PCR). A control group of ten patients with sporadic gastric cancers in the upper third of the stomach was also similarly analyzed.

Results. MSI was demonstrated in eight of nine cancers from the individuals who had had primary gastric cancer or gastroduodenal ulcer (88.9%) compared with two of ten cancers from the individuals with sporadic gastric cancer in the upper third of the stomach (20%).

Conclusion. These results suggest that one or more MSI is associated with remnant gastric cancer after gastrectomy.

Key words: microsatellite instability, gastric remnant cancer, replication error, malignant potential, remnant stomach

Introduction

The incidence of cancer of the gastric remnant has steadily increased [1–5], and this increase may be related to the high frequency of gastrectomies performed 20 to 30 years ago. Many reports have studied the pathogenesis and causes of gastric remnant cancer [5–10], but there is little information available on molecular biological analyses to identify individuals susceptible to gastric remnant cancer.

A subset of gastrointestinal cancers harbors numerous genetic alterations which are often found in simple

repeated DNA sequences known as microsatellite DNA [11]. This phenotype has been termed microsatellite instability (MSI) or replication error, and is almost always a feature of cancers in patients who have hereditary non-polyposis colorectal cancers (HNPCC) [12,13]. This phenotype leads to a dysfunctional DNA mismatch repair system in the cancers. A defective mismatch repair can lead to a cascade of secondary mutations, some of which may affect both oncogenes and tumor suppressor genes and thus give rise to cancer [14,15].

The aim of this study was to clarify whether one or more MSI is associated with remnant gastric cancer after gastrectomy.

Patients and methods

Nine patients with gastric remnant cancer who had undergone total gastrectomy at the First Department of Surgery of the Ryukyu University Hospital between 1984 and 1995 were included in this study. Gastric remnant cancer was defined by the following criteria: (1) initial diagnosis of primary gastric cancer or primary gastroduodenal ulcer; (2) in the patients with primary gastric cancer, a radical gastrectomy (surgically, regional nodal dissection and proximal clearance margin greater than 5 cm from the tumor edge; histologically, no tumor at the resection margin) should have been performed; and (3) the time between the primary gastric resection and the occurrence of gastric remnant cancer exceeded 5 years.

Of the nine patients, four had undergone a gastrectomy for primary gastric cancer, and five had undergone a gastrectomy for benign gastroduodenal disease (ulcer); one had an (end-to-end) gastroduodenostomy (Billroth I gastrectomy) and four had (end-to-side) gastrojejunostomies (Billroth II gastrectomy), while the four patients with primary gastric cancer had gas-

trooduodenostomies. The patients consisted of seven men and two women. The mean age of the patients at the time of diagnosis of gastric remnant cancer was 67.2 years (range, 55 to 48 years). The mean time between the primary gastric resection and the occurrence of gastric remnant cancer was 30.4 ± 4.9 years (range, 20 to 42 years) for the patients with primary gastroduodenal ulcer and 12.5 ± 5.0 years (range, 5 to 22 years) for those with primary gastric cancer.

Ten patients with primary gastric cancer of the upper third of the stomach who underwent a radical gastric resection in the same period served as a control group. They consisted of nine men and one woman, with a mean age of 65.2 ± 7.9 years (range, 38 to 78 years). No patients had a family history of HNPCC. Table 1 shows the tumor characteristics (depth of invasion and tumor stage) of the gastric remnant cancer and cancer in the controls. There were no differences in the tumor characteristics between the two groups. The histologic type of cancer also showed no differences between the two groups.

Cancers and corresponding normal tissues were fixed with formalin and embedded in paraffin. Using a hematoxylin-eosin-stained section as a guide, we microscopically dissected serial 8- μ m sections from these samples to separate the tumor from normal tissues. DNA was extracted from the regions that contained at least 80% of tumor cells or normal cells. Genomic DNA was extracted by the standard proteinase K and phenol-chloroform methods.

Microsatellite analysis

We used oligonucleotide primer sets for the six microsatellite loci containing cytosine-adenine dinucleotide repeats to observe any replication errors (RER); D1S213, D8S135, D10S197, D17S518, D17S807, and D18S34. Genomic DNA (50 to 100 ng) was used as a template for polymerase chain reaction (PCR) amplification in 50- μ l reaction volumes. Amplification was performed by the hot start method, using Taq polymerase (TaKaRa Taq; TaKaRa, Kyoto, Japan) and Taq start antibody (Clontech Laboratories, Palo Alto, CA, USA) in a DNA thermal cycler (Perkin-Elmer, Foster City, CA, USA), using 35 amplification cycles, consisting of 1 min at 94°C , 1 min at 56°C , and 1 min at 72°C . The PCR buffer included final concentrations of 10 mM Tris-HCl, pH 8.3, 1.5 mM MgCl_2 , 50 mM KCl, and each dNTP at 250 μ M.

The PCR products and denaturing stop solution (95% formamide, 10 mM ethylenediaminetetraacetic acid [EDTA], 0.25% bromophenolblue, and xylene cyanol FF) were heated at 95°C for 5 min and then rapidly cooled on ice. Electrophoresis was carried out on 10% polyacrylamide gels at 200 V for 6 h, and the gels were stained with silver (Silver Stain Kit; Daiichi Pure Chemicals, Tokyo, Japan).

For the detection of MSI, the PCR products of tumor and normal DNA from the same patients were run in adjacent lines, and the patterns were compared. MSI was defined as positive when the tumor DNA displayed

Table 1. Tumor characteristics in the gastric remnant group and control group

	Gastric remnant group	Control group
Age, in years (range)	67.2 ± 8.0 (55–84)	65.2 ± 7.9 (38–78)
Male:female	7:2	9:1
Depth of invasion		
m	2	1
sm	3	1
mp	0	0
ss	0	2
se	2	6
si	2	0
Stage ^a		
Ia	4	1
II	0	0
IIIa	1	3
IIIb	0	2
IVa	0	3
IVb	4	1

m, Mucosa; sm, submucosa; mp, muscularis propria; ss subserosa; se (serosa-exposed), tumor invasion observed on the serosal surface and the peritoneal cavity; si (serosa-infiltrating), tumor invasion observed in adjacent structures

^aThe stage was classified according to the Japanese Classification of Gastric Carcinoma

fragment sizes not present in normal DNA. The PCR products were purified using the QIA-quick spin PCR purification kit (Qiagen, Chatsworth, CA, USA) and then directly sequenced (TaKaRa), using the end-labeled primers and kit protocols of the manufacturer.

Statistical analysis

The χ^2 test was used to determine significance regarding the microsatellite instability data.

Results

Figure 1 shows the typical polyacrylamide gel findings of gastric DNA amplified by PCR. The MSI data are summarized in Table 2.

In the gastric remnant cancer samples (patients 1–9), eight of the nine patients (88.9%) showed MSI at one locus. In one of them (11.1%), MSI was detected at two loci. This patient had undergone gastrectomy for the primary gastric cancer. When analyzed by the type of reconstruction, MSI was detected in four of the five patients with gastroduodenostomy and in four of the four patients with gastrojejunostomy. The time between the primary gastric resection and the occurrence of gastric remnant cancer was not significantly different between patients with MSI-positive cancer (22.7 years) and those with MSI-negative cancer (22 years).

In the control group (patients 10–19), two of the ten cancers (20%) were found to be unstable. Both showed MSI at two loci. The histologic type of cancer and MSI were not related in either group.

The difference in MSI frequency between the gastric remnant cancer and control groups was highly significant ($P < 0.01$) (Table 3).

Discussion

In this study, the frequency of MSI was investigated in nine gastric remnant cancers. It was found that eight of these cancers (88.9%) showed MSI. In the control group of patients who had only one cancer in the upper third of the stomach, MSI occurred in only two of the ten patients (20%). In studies concerning MSI in gastric cancer, the incidence of MSI-positive cancers ranged from 15% to 39% [13,16–19]. In this study, the difference in the frequency of MSI between cancers from the gastric remnant group and those from the control group was significant. This finding suggests that gastric remnant cancer may be rare, but that patients with a remnant stomach tend to develop metachronous gastric cancer more often than patients with sporadic gastric cancer in the upper third of the stomach.

As for MSI, there are two problems regarding its definition [19]: (1) How many altered microsatellite loci are required for the condition to be classified as MSI?

Table 2. Microsatellite instability in the gastric remnant group (patients 1–9) and control group (patients 10–19)

Patient number	Microsatellite loci					
	D1S213	D17S518	D18S34	D17S807	D8S135	D10S197
1	–	–	–	+	–	–
2	–	–	–	+	–	–
3	–	–	–	+	–	–
4	–	+	–	–	–	–
5	–	–	–	+	–	–
6	+	–	+	–	–	–
7	–	–	–	–	–	–
8	–	+	–	–	–	–
9	–	–	–	+	–	–
10	–	–	–	–	–	–
11	–	–	–	–	–	–
12	+	–	–	–	–	+
13	–	–	–	–	–	–
14	–	–	–	–	–	–
15	–	–	–	–	–	–
16	+	–	+	–	–	–
17	–	–	–	–	–	–
18	–	–	–	–	–	–
19	–	–	–	–	–	–

+, Microsatellite instability (MSI) (+); –, MSI (–)

Table 3. Microsatellite instability in the gastric remnant group and control group

	One locus (+)	Two loci (+)	Total
Gastric remnant group ($n = 9$)	7/9 (77.7)	1/9 (11.1)	8/9 (88.9)
Control group ($n = 10$)	0/10 (0.0)	2/10 (20)	2/10 (20)

The difference in the frequency of MSI between the gastric remnant group and the control group is significant ($P < 0.01$)

The values in parentheses are percentages

Fig. 1. Representative example of a microsatellite instability (MSI) test at D17S518. *N* indicates DNA from normal tissue; *T* indicates DNA from the tumor. *Lane a*, Patient 10; *lane b*, patient 8; *lane c*, patient 5. MSI-positive carcinoma (*lane b*) shows deviation from the normal pattern [16]

and (2) how many microsatellite loci, and which loci, should be examined to determine MSI? Unfortunately these two problems have not yet been resolved. In accordance with most studies that examined MSI [13, 16–19], one or more altered microsatellite loci were classified as MSI; we examined six microsatellite loci in

this study. As the definition of MSI has not been standardized among researchers, a new proposal for the criteria of MSI is thus expected.

The pathogenic factors responsible for the transformation of the mucosa in the remnant stomach to dysplasia and cancer remain unclear. They are multiple and include hereditary factors, infection with *Helicobacter pylori*, the action of carcinogenic N-nitroso compounds, and continued reflux injury [5]; these factors are considered to induce atrophic gastritis, intestinal metaplasia, and dysplasia. Dysplasia, especially high-grade dysplasia, in a given patient may not only be a marker of a heightened cancer risk but may itself already be associated with early cancer elsewhere. In addition, it is also important to clarify the mechanism of this carcinogenesis to assist in the diagnosis and treatment. However, whether or not DNA instability contributes to an accumulation of genetic alterations of known multiple tumor-suppressor genes and oncogenes still remains to be elucidated.

In sporadic colorectal cancer, the frequency of MSI (RER)-positive tumors was 3% in adenoma and mucosal carcinoma [20]. It increased in invasive carcinoma and then showed a marked increase in carcinoma which metastasized to the liver. These studies suggest that DNA instability may contribute to additional alterations necessary for the progression of tumors to a highly malignant stage, such as metastasis [21]. Similarly, an abnormal nuclear DNA content, cell cycle abnormalities, or a high expression of oncogenes or tumor-suppressor genes are common in dysplasia and cancer of the stomach [5,22,23]. These findings indicate that the gastric mucosa, including the remnant mucosa, is prone to genomic instability, and some of these patients with genomic instability develop cancer. In oncogene studies, some genes may thus be a helpful marker for identifying dysplastic lesions that tend to progress to cancer. However, the potential clinical utility of specific oncogenes in predicting a heightened cancer risk remains to be clarified.

In conclusion, this study demonstrated that MSI is a common feature of cancers in patients who develop gastric remnant cancer. It was detected in 88.9% of gastric remnant cancers in these patients. These findings suggest that the presence of one or more MSI

is associated with remnant gastric cancer after gastrectomy.

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