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Case report

Gastritis cystica polyposa associated with a gastric stump carcinoma, with special reference to cell kinetics and *p53* gene aberrations

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Abstract

We report a case of gastritis cystica polyposa (GCP) that developed in association with a small stump carcinoma. The patient had had distal gastrectomy for peptic ulcer 33 years prior to the present illness. Total gastrectomy was carried out for the stump carcinoma of the remnant stomach, followed by Roux-en-Y anastomosis. Histological examination revealed that the cancer was associated with a GCP lesion in its neighborhood. The resected stomach was subjected to a cell kinetics study and *p53* gene analysis, as GCPs are thought to have a high potential for carcinogenesis. The GCP mucosae, as well cancer tissues and remnant mucosae obtained from the same specimens, were investigated and compared. We found that cell kinetics, as measured by a Ki-67 labeling index count, was more accelerated in the GCP than in the remnant mucosa, and that *p53* gene aberrations, including both mutations and deletions, took place in the GCP lesion. As the *p53* gene is considered to be recessive, in principle, its tumor suppressive activity is lost only when gene aberration, either mutation or deletion, occurs concurrently or successively in both alleles. It was of interest to us that a benign lesion such as GCP had, in this instance, already developed both gene aberrations, strongly suggesting a precancerous nature for this disease.

Key words Gastritis cystica polyposa · Ki-67 LI · *p53* Gene mutation · *p53* Gene deletion

Introduction

Gastritis cystica polyposa is a unique lesion found on occasion at the stoma of a gastrojejunal anastomosis a long time after distal partial gastrectomy. The pathological criteria were first described by Nicolai and Mueller [1] in 1965, and the term "gastritis cystica polyposa (GCP)" was proposed by Littler and Gleibermann [2] in 1972. Later, in 1979, Koga et al. [3] named the same lesion stomal polypoid hypertrophic gastritis (SPHG), and both terminologies are equally prevalent today. Koga et al. [3] described the histological features of the lesion as represented by the elongation of gastric pits, hyperplasia and cystic dilatation of the pseudopyloric glands, and their invasion to the submucosal layer.

Many cases of gastric stomal cancer, found in association with GCP, have now been reported [4–6], and a high potential for carcinogenesis in GCP mucosa has been suggested. However, little basic study has been done, and the mechanism of such potential carcinogenesis is not yet clear.

We encountered a patient with a GCP lesion at the stoma of the remnant stomach, associated with a small carcinoma. We carried out studies of cell kinetics and *p53* gene aberrations in the cells of the GCP mucosa in specimens obtained from the patient's resected stomach. Cancer tissues and non-cancerous-non-GCP mucosae of the remnant stomach (remnant mucosa) were also studied for comparison.

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Case report

A 60-year-old man who had had Billroth-II surgery 33 years previously had epigastric distress of 1-week duration when he visited a doctor. He underwent an upper gastrointestinal (UGI) series and was referred to our hospital after being diagnosed with a stomal tumor. Endoscopy revealed that reddened and hypertrophic mucosa at the lesser curvature side of the stoma, forming a sessile polypoid elevation, and having continuity to the neighboring mucosal folds (Fig. 1). A small ulceration was found in the center of the elevated lesion. Biopsy samples taken from the ulcerated portion showed adenocarcinoma, but those taken from the rest of the elevated lesion were diagnosed as benign hyperplastic epithelium.

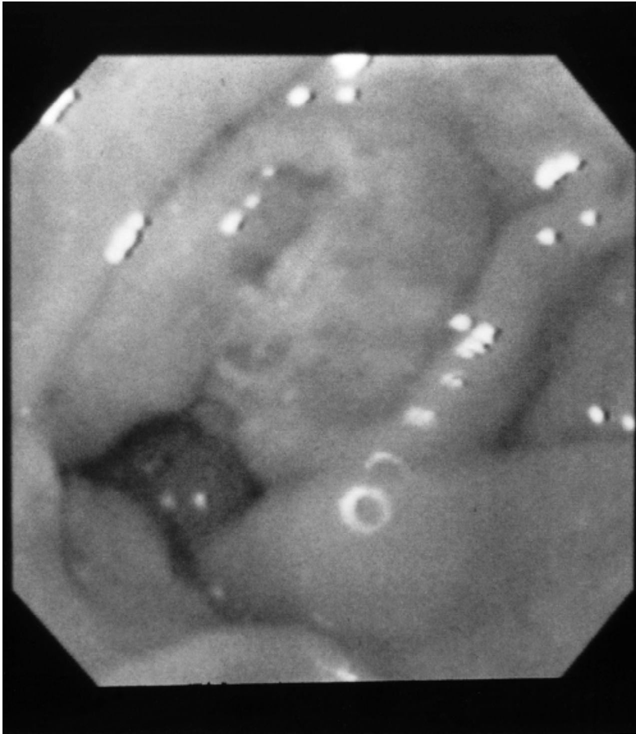

Fig. 1. Endoscopic findings. Endoscopy revealed a sessile elevation of the mucosa, with an ulceration in its center, at the lesser curvature side of the stoma

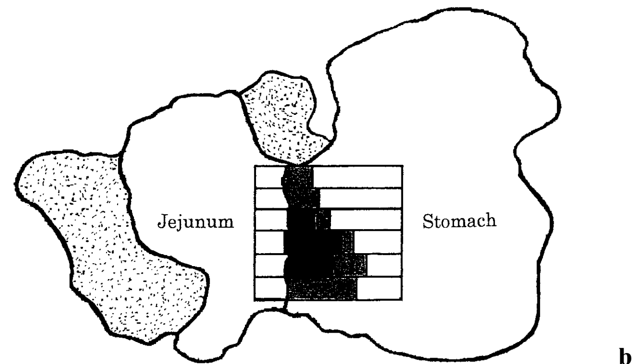

Fig. 2a,b. Resected stomach; **a** photograph; **b** histological mapping — a comparison. Reddened and swollen mucosa was noted zonally along the suture line, with an irregularly shaped small ulceration inside (**a**) the lesions of the gastritis cystica polyposa (GCP); *gray area* and the carcinoma (*black area*), (**b**)

An operation was performed for a gastric cancer that had developed adjacent to the suture line, associated with localized hyperplastic gastritis. Total resection of the remnant stomach was carried out, followed by Roux-en-Y anastomosis.

Pathological examination revealed that the cancerous lesion was a well differentiated adenocarcinoma invading the proper muscle layer. Nodal metastases were not present. In the localized hyperplastic mucosa, elongation of the pits was remarkable. Hyperplasia and cystic dilatation of the pseudopyloric glands and their invasion to the submucosal layer were also conspicuous. Fundic glands were atrophied or had disappeared. All of these histological features were compatible with the diagnosis of GCP (Figs. 2, 3).

The patient's postoperative course was uneventful, and he was discharged 30 days postoperatively.

Determination of cellular proliferative activity

Using specimens obtained from formalin-fixed and paraffin-embedded blocks of the resected stomach, cellular proliferative activity was investigated by immunohistochemical staining of Ki-67 antigen. Cell kinetics in each of the tissues were determined by noting the proportion of proliferating cells among all epithelial or cancer cells by calculating the Ki-67 labeling indices (LIs). Ki-67 staining was carried out by the streptavidin-biotin method, using MIB-1 antibody (Immunotech, Marseille, France). In the GCP and the remnant mucosa, 20 pits cut longitudinally from the surface to the bottom, including the generative zone, were selected in each section of specimen to make the count as accurate as possible. As for the cancer tissues, 20 of the most differentiated portions of the lesion that formed tubule-like structures were selected for the same purpose. The Ki-67 LIs were 69.8 ± 13.8 in the cancer tissue, 66.3 ± 10.6 in the GCP, and 18.5 ± 8.9 in the remnant mucosa. The Ki-67 LI in the GCP was as high as that in the cancer tissue, and significantly higher ($P < 0.01$) than that in the remnant mucosa. The distribution of positively stained cells was both dense and diffuse in the cancer tissues as well as the GCP mucosa. However, in the remnant mucosa, they were observed only in the bottom of the pits and in the generative zone (Fig. 4).

Overexpression of *p53* gene products in the nuclei

Immunohistochemical staining for p53 protein was conducted with anti-p53 monoclonal antibody (DO 7; Nova Castra, NewCastle, UK) by the streptavidin-biotin method to determine abnormal accumulation of *p53* gene products in the nuclei. In this study, the nuclei of the cancer cells were stained positive in both a diffuse and a dense manner, but in the GCP lesion, only small numbers of positively stained cells were noted. These were localized to the middle of the mucosal layer, which could correspond to the generative zone of the normal mucosa. No stained cells were observed in the remnant mucosa.

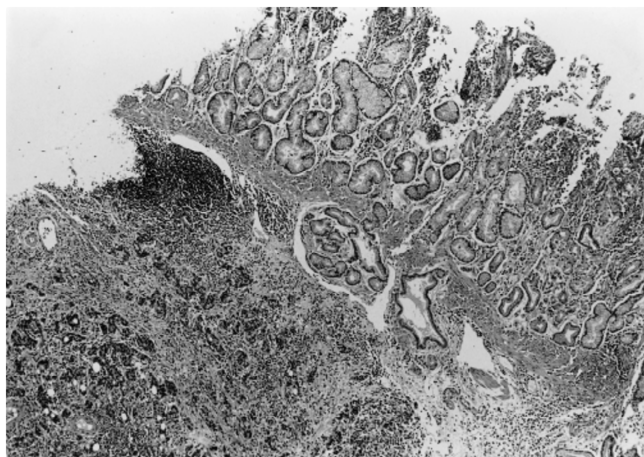

Fig. 3. Histological findings. Histological view of the ulcer edge, showing carcinoma invading underneath the GCP mucosa. Massive stromal infiltration of lymphocytes was noted in the carcinoma. H&E, ×40

p53 Gene mutation

To detect the occurrence of *p53* gene mutations, a polymerase chain reaction single-strand conformation polymorphism (PCR-SSCP) method was used. The targeted tissues; the carcer, the GCP, and the remnant mucosa, were selectively scraped out manually under a stereomicroscope from a 10-μm-thick slice of tissue block. DNA in each tissue was extracted using a Sepa-gene kit (Sankyo Junyaku, Tokyo, Japan). Mutations of the *p53* gene were determined using nested PCR for exons 5,6,7, and 8, and the PCR products were electrophoresed and identified by autoradiography. From this analysis, abnormally mobilized DNA bands were found in the GCP mucosa — sited at exon 6 of the *p53* gene — but not in the cancer or in the remnant mucosa. Following this result, DNA fragments were scraped out from the abnormally mobilized bands on the SSCP gel, and direct DNA sequencing of the samples was conducted using Sequennase Version 2 (US Biochemical, Cleveland, OH, USA). This procedure revealed that

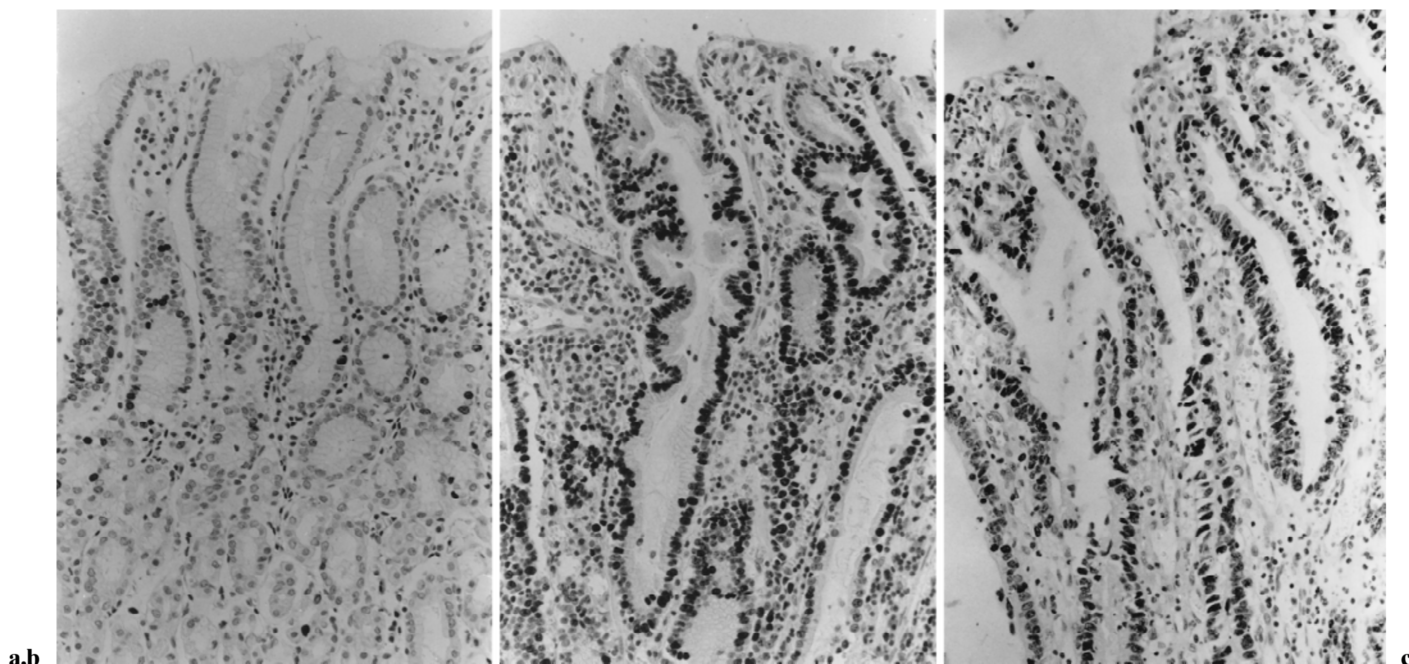

Fig. 4a–c. Immunohistochemical staining of Ki-67. MIB-1 staining showed positive cells located only at the bottom of the pits and in the generative zone in normal mucosa (**a**), but spread over all tubules in the GCP (**b**) and in the carcinoma (**c**)

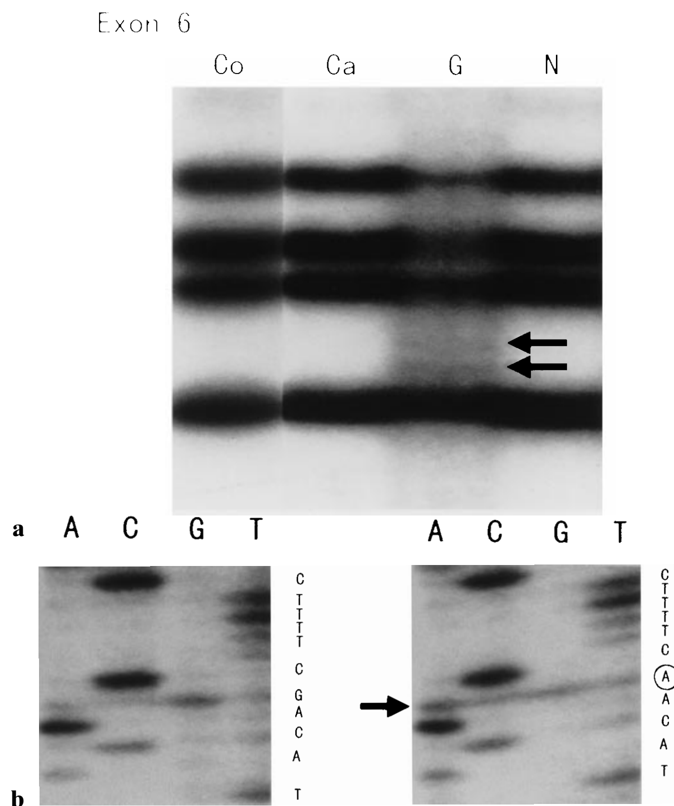

Fig. 5a,b. Single-strand conformation polymorphism (SSCP) method and direct sequencing for *p53* mutation. Abnormally mobilized bands (*arrows*) were detected on the gel in exon 6 of the *p53* gene in the sample obtained from the GCP tissues (**a**). *Co*, Control (lymphocyte); *Ca*, carcinoma; *G*, GCP; *N*, remnant Mucosa. **b** The mutation was found to be a substitution from arginine (CGA) to glutamine (CAA), (*arrow*) by a direct sequencing procedure conducted subsequently

this mutation occurred at codon 213 of exon 6 and was a substitution from arginine (CGA) to glutamine (CAA) (Fig. 5).

p53 Gene deletion

The occurrence of *p53* gene deletions in each tissue was investigated. For this analysis, a double-targeted fluorescence in situ hybridization (FISH) method was employed, using a combination of a probe for the *p53* cosmid labeled by digoxigenin, and a biotinized probe for chromosome 17 centromere. Two-color FISH analysis was carried out according to the instructions of the probe's manufacturer (Oncor, Gaithersburg, MD, USA). In the fluorescence dyeing, anti-fluorescein isothiocyarate (FITC) avidin (Boehringer, Indianapolis, IN, USA) was used for the centromeric probe, and anti-digoxigenin rohdamine (Vysis, Downers Grove, IL, USA) was used for the *p53* probe. Copy numbers of the

No. of p53 Rhodamine signals \ No. of 17-centromere FITC signals	0	1	2	3	4	5 ≦	Total
0							
1		21	31				52
2			48				48
3							
4							
5 ≦							
Total		21	79				100

Fig. 6. Two-color fluorescence in situ hybridization (FISH) analysis of GCP cells. Among 100 GCP cells, 21 had monosomy and 31 had deletions of the *p53* locus, resulting in the proportion of *p53* gene deletion being 52%. *FITC*, Fluorescein isothiocyarate

p53 and the centromeric signals in 100 nuclei per specimen were then counted under a fluorescence microscope, following the criteria advocated by Hopman et al. [7]. A deletion of the *p53* gene represented a copy number of *p53* signals less than that of the centrometric signals of chromosome 17, or monosomy of chromosome-17 itself. The proportion of deletions of the *p53* locus in 100 nuclei per specimen was thus calculated.

By this analysis, the proportion of cells with *p53* gene deletion was 52% in the GCP (Fig. 6), whereas it was just 14% in the cancer tissue, and only 10% in the remnant mucosa.

Discussion

Gastritis cystica polyposa (GCP) is a unique type of chronic gastritis represented by a sessile elevated lesion along a suture line, occurring a long time after Billroth-II surgery or simple gastrojejunal anastomosis. Recently, this lesion has begun to attract the interest of researchers in respect to its relationship to carcinogenesis. Many of the GCP lesions reported in the past [4–6] were found in association with early or small cancerous lesions which had developed just inside or adjacent to the GCP mucosa. It is commonly accepted that the reason GCPs develop almost exclusively at the stoma, is that the pathogenesis of this lesion is derived from the duodeno-gastric reflux (DGR) [8], so that most reported GCPs are found in remnant stomach that had had Billroth-II surgery.

In Western countries, it is widely accepted that a remnant stomach after partial gastrectomy for peptic ulcer disease has an increased risk of developing a

carcinoma, with a latency of more than 20 years [9–14], the risk being three; to fivefold higher than that in a matched population group [10]. It has also been widely recognized [10,15] that treatments with Billroth-II or simple gastrojejunostomy lead to higher risks of the development of stump carcinomas than those with Billroth-I or other non-reflux types of anastomosis.

These clinical concepts have been supported by many animal studies. In 1981, Langhans and colleagues [16] first documented stump carcinomas developed in rat stomach by making a DGR type of gastrointestinal anastomosis without any assistance of carcinogenic agents. After that, many animal studies were conducted, using similar types of rat models, and consequently these supported Langhans' results [17–19]. Interestingly, in these rat models of DGR, hyperplastic and cystic epithelia, resembling human GCP mucosa, were commonly found [17,20]. When these findings are considered, it may be reasonable to speculate that there are close relationships among DGR, GCP, and gastric stump carcinomas.

However, very little basic analysis of carcinogenesis in the mucosa of GCP has been reported. Bechi and colleagues [21] reported that the ^{3}H-thymidine labeling indices of stomal mucosa after Billroth-II were significantly higher than those after Billroth-I or Roux-en-Y surgeries. Jaszewski et al. [22] reported that ornithine decarboxylase activity in the gastric mucosa of patients with Billroth-II reconstruction was much higher than the activity in patients with Billroth-I. Yokoyama and Hattori [23] reported a patient with a GCP lesion that showed abnormal accumulations of p53 protein in the nuclei of the cystically dilated tubules, and they referred to the carcinogenic potential of the lesion.

We found that the cell kinetics in the GCP mucosa was greatly accelerated compared with that in the remnant mucosa, and that in the GCP mucosa it was as high as that in the cancer tissues. We thought these phenomena could arise from regulatory failure in the cell cycle.

As *p53* is a recessive gene, it is generally accepted that its tumor suppressive activity is lost when gene aberrations occur concurrently or successively in both alleles, and the aberrations are usually mutation in one allele and deletion in the other. When its activity is lost, the regulatory mechanism of the cell cycle is damaged, resulting in uncontrolled cell proliferation. In the patient reported here, we found abnormal accumulations of p53 protein, a point mutation, and deletions of the *p53* gene in the GCP mucosa. However, the reason that these aberrations of the *p53* gene were more prominently detected in the GCP than in the cancer tissues is not known. In the cancer tissues, expression of p53 protein was dominant. Nevertheless, *p53* gene mutation was not detected. In the literature [24], it has been reported that, in various cancers showing overexpression of p53 protein, *p53* gene mutation was detected in 84.6% in exons 5–8, and in the remainder, *p53* gene mutation was not detected despite positive staining for p53 protein in these exons. Interpretation of this discrepancy is difficult, but we think the possibility should be considered that the mutations may have occurred in exons that have not been examined. The discrepancy found in our patient may be explained similarly. It is also difficult to explain why the incidence of *p53* gene deletion in cancer tissues was lower than that in GCP tissues. We think that a considerably large number of signals from intermingled lymphocytes, as well as those from cancer cells may have been counted, because the cancer lesion in our patient had massive stromal infiltration of lymphocytes, as shown in Fig. 3.

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