Material and methods

Study area

The study was conducted in a town located in a rural area of Hiroshima Prefecture, Japan, with a total population of approximately 4500 (male/female ratio: 0.91), 39.3% of the population being over 65 years of age. The town was chosen for this study because gastric cancer screening by serum PG concentration testing had been conducted there since 1989. Data pertaining to diagnosis of gastric cancer were obtained from the death certificate records and the Hiroshima Prefectural Cancer Registry. Individuals residing in the town during the relevant time period were selected at the town office. The study was approved by the ethics committee of Hiroshima University School of Medicine and by the Ministry of Public Management, Home Affairs, Posts and Telecommunications of Japan.

Screening by serum pepsinogen testing

Fasting blood samples were obtained from the participants, and serum samples were stored at −20°C until use. Serum PG I and II were measured by radioimmunoassay (Dainabot, Tokyo, Japan) [18,19]. Screening results were determined according to established cut-off values: individuals with a value less than the cut-off value were considered to be at high-risk for gastric cancer and were advised to undergo a more specific examination. The cut-off levels during the study period were as follows: a PGI/PGII ratio of less than 2 in 1989; a PG I concentration of less than 30 ng/ml or a PGI/PGII ratio of less than 2 in 1990–92; a PG I concentration of less than 50 ng/ml and a PGI/PGII ratio of less than 3 after 1993, as reported previously [15,18,19]. The screening was offered annually in the town through the local government. The target population comprised all residents aged 40 years or older. In 2002, there were 1596 residents in the town aged 40 years or older and 663 (41.5%) individuals participated in the gastric cancer screening. During 13 years, 873 residents had positive screening tests by serum pepsinogen and 585 (67.0%) underwent more specific examinations (gastointestinal endoscopy; 539 (92.1%), photofluorography; 46 (7.9%)). Some of the results were reported previously [15,18,19].

Identification of individuals who died from gastric cancer

The case series consisted of residents of the town, who, according to the death certificate records and the Hiroshima Prefectural Cancer Registry, died from gastric cancer during the period April 1989 to March 2002. Forty-nine individuals died from gastric cancer during the period. Gastric cancer death was confirmed in 46 (93.9%) of these individuals (28 M, 18 F). Of the 46 deaths, 5 were prior to the initiation of serum PG testing; thus, 41 cases were finally selected for the study.

Identification of control subjects

For each of the 41 individuals who died of gastric cancer, 3 gender-matched control subjects with the same birth year were selected randomly from the residents that were alive on the date that the individual died. When there were fewer than three other residents with a matching birth year, the matching was done with residents whose birth year was within ±3 years. For the 41 individuals who died from gastric cancer, a total of 123 control subjects were selected.

Identification of screening history

Records of the gastric screening program were reviewed to obtain screening histories for the 41 individuals who died and the 123 control subjects during the 2 years before each diagnosis of gastric cancer.

Data analysis

The Mantel-Haenszel procedure was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs). ORs were calculated for control subjects who participated in the screening once during the 1 or 2 years before case diagnoses, in comparison with those who did not participate in the screening during this period. The relation between participants' age and reduction in deaths from gastric cancer was examined. In the analysis, age was categorized as less than 65 years, less than 70 years, less than 75 years, less than 80 years, and less than 85 years.

Results

Age and gender of the individuals who died from gastric cancer and the control subjects are reported in Table I. The male-to-female ratio of the individuals who died was 25/16, and the mean age of these individuals was 71.9 years (range, 44–92 years).

The ORs for death from gastric cancer among control subjects who participated in the screening once during the 1 year and once during the 2 years before the diagnosis of cases, as opposed to death from gastric cancer among those who did not participate in the screening during these periods,

Table I. Age and sex of individuals who died from gastric cancer and control subjects.

	Case ($n=41$)	Control ($n=123$)
Age (years) at diagnosis		
40–59	3	10
60–69	15	31
70–79	9	52
80–	14	30
Male/female ratio	25/16	75/48

Table II. Mantel-Haenszel odds ratios for death from gastric cancer in PG testing performed during 1 year before gastric cancer diagnosis.

	Number of screening participants (out of 3 matched controls) during the year before gastric cancer diagnosis			
	0	1	2	3
Gastric cancer deaths				
Screening history	1	1	0	0
No screening history	22	14	2	1

were 0.238 (95% CI: 0.061–0.929) and 0.375 (95% CI: 0.156–0.905), respectively (Tables II and III). Thus, the number of gastric cancer deaths was reduced by 76% among subjects participating in PG screening within 1 year and 62% among subjects participating in PG screening within 2 years of the time of gastric cancer diagnosis in the corresponding individuals who died from gastric cancer. The OR for death from gastric cancer among subjects participating in screening once during the 1 year before the diagnosis of cases was lower than that of subjects who participated once during the 2 years before diagnosis.

The reduction in the number of deaths from gastric cancer did not correlate with age of subjects who underwent screening once during the 1 year before diagnosis of the cases (Table IV). The reduction was not related to age in any subjects with the exception of those less than 85 years of age who underwent screening once during the 2 years before diagnosis of the cases (OR: 0.321; 95% CI: 0.120–0.862; $p=0.027$).

Discussion

This is the first case-control study evaluating the relation between mass screening on the basis of PG concentration and gastric cancer mortality. In Japan, mass photofluorography screening programs for gastric cancer were initiated around 1960 [1]. The usefulness of such screening has been reported. A

Table III. Mantel-Haenszel odds ratios for death from gastric cancer in PG testing performed during 2 years before gastric cancer diagnosis.

	Number of screening participants (out of 3 matched controls) during 2 years before gastric cancer diagnosis			
	0	1	2	3
Gastric cancer deaths				
Screening history	3	1	1	0
No screening history	15	13	5	3

Abbreviation: PG = pepsinogen.

population-based, case-control study of screening based on photofluorography showed a 55% reduction in gastric cancer deaths for individuals who participated in the screening at least once during a 2-year period [1–4]. Lee et al. reported a 2-fold decrease in gastric cancer mortality in subjects who participated in photofluorography screening during the preceding 1 year versus control subjects [5]. At present, about 5 million people in Japan are screened annually by photofluorography.

The sensitivity of photofluorography is by no means high when endoscopy is used as a yardstick. Thus, measurement of serum PG concentrations has recently gained attention as a new screening method for gastric cancer. The results of studies of PG concentration testing show that this method has a superior cancer detection rate and is less expensive than the conventional photofluorography-based mass screening [21,22]. Furthermore, the percentage of early gastric cancers detected by PG testing is higher than that of conventional screening, and a considerable number of individuals with gastric cancer identified by PG testing have been treated by endoscopic surgery. Miki et al. reported the incidence of gastric cancer to be 0.05% by X-ray detection and 0.18% by PG testing in populations who underwent X-ray and PG screening simultaneously, and that 90% of gastric cancers detected by the PG method were in the early stages [21]. It has been reported that screening by serum PG testing may be useful in detecting small asymptomatic cancer of non-ulcerated morphology, and well-differentiated histology [1]. Small asymptomatic cancers of this type are relatively difficult to detect by photofluorography, whereas such conventional screening is effective in detecting cancers of ulcerated morphology and poorly differentiated histology as well as advanced cancers, which are frequently symptomatic. In addition, the PG method has many advantages over the X-ray method. For example, PG testing is easy to perform, and patients do not feel much discomfort. There is no radiation exposure,

Table IV. Reduction in gastric cancer mortality and age.

Age (years)	*n*	Participation in screening during 1 year before gastric cancer diagnosis in 3 matched controls			Participation in screening during 2 years before gastric cancer diagnosis in 3 matched controls		
		OR	95% CI	*p*-value	OR	95% CI	*p*-value
<65	11	–	–	0.562	0.600	0.081–4.444	>0.999
<70	18	–	–	0.188	0.385	0.094–1.567	0.166
<75	25	0.429	0.056–3.726	0.444	0.333	0.096–1.156	0.146
<80	30	0.500	0.100–2.495	0.514	0.500	0.163–1.532	0.306
<85	36	0.294	0.072–1.209	0.161	0.321	0.120–0.862	0.027
All	41	0.238	0.061–0.929	0.043	0.375	0.155–0.905	0.036

Abbreviations: OR = odds ratio; CI = confidence interval.

and there is no barium ingestion, with its related side effects. The PG method is fast, and many serum samples can be analyzed simultaneously. If man-power, costs, and efficiency were not issues, the highest sensitivity could be reached in all individuals by using a combination of PG and photofluorograhy screening. However, since man-power and cost are limiting factors, and photofluorography produces some harmful effects, the more efficient screening method should be determined on the basis of scientific and epidemiologic evidence.

There have been no studies directly examining whether screening on the basis of the serum PG concentration is associated with reduced gastric cancer mortality. A randomized, controlled trial is the most suitable method for evaluating the effectiveness of a cancer screening program; however, mass screening programs for gastric cancer have been so widely implemented in Japan that it is impossible to undertake such a trial. As an alternative, we undertook this case-control study.

Because the screening histories of the individuals who died from gastric cancer and the control subjects in the present study were obtained from the same data source, recall bias and inter-observer bias were thought to be eliminated. Measurement bias was also eliminated because the record linkage on the computer was performed similarly for cases and control subjects. However, our study had two weaknesses. First, the self-selection bias could not be controlled because some information could not be obtained. If a family history of cancer increased the risk of death from gastric cancer, the effectiveness of the screening might have been underestimated if this factor was not taken into account. Similarly, if habitual smoking increased the risk, the effectiveness might have been overestimated. In fact, in several studies it is suggested that the risk of gastric cancer may be associated with a family history of cancer and with habitual smoking [23,24]. These factors and other factors, such as socio-economic status, dietary habits, and health practices, should be controlled in order to evaluate the screening method more precisely. Another consideration is the effect of mass X-ray screening. The effect was not evaluated in the present study because we could not sufficiently investigate the subjects' histories of X-ray screening.

The present study indicated that the effect of screening based on the serum PG concentration lasts for at least 2 years. Fukao et al. reported that the effect of screening by photofluorography remains for at least 5 years [3]. We previously reported that, in more than 90% of cases, the PG concentration was similar to that obtained 5 years previously [25]. We investigated the natural course of the PG concentration in a prospective study over a period of 9 years. Of 207 PG-negative subjects, 182 (87.9%) were still PG negative after 9 years [26]. Therefore, repeated examination of the PG concentration might not be required. However, the optimal interval between PG tests should be decided by the effectiveness of the screening method. Further study is necessary to confirm the optimal interval.

In conclusion, the results of the present case-control study suggest reductions of 76% and 62% in gastric cancer mortality among individuals who participate in screening for gastric cancer on the basis of the PG concentration once a year or once every 2 years, respectively. A large-scale study including factors related to self-selection bias is needed not only to evaluate the effectiveness of screening based on PG testing more precisely but also to determine the optimal frequency and interval of screening by this method.

Acknowledgements

We thank Drs Kazumasa Miki (Division of Gastroenterology and Hepatology, Toho University School of Medicine, Tokyo, Japan), Koji Sumii (Saiseikai Hiroshima Hospital, Hiroshima, Japan), and our colleagues in Hiroshima University Hospital, the

town office staffs, and the Hiroshima Prefectural Cancer Registry for their cooperation. This work was supported in part by a Grant-in-Aid from the Ministry of Health, Labor and Welfare of Japan.

References

[1] Tsubono Y, Hisamichi S. Screening for gastric cancer in Japan. Gastric Cancer 2000;3:9–18.

[2] Oshima A, Hirata N, Ubukata T, Umeda T, Fujimoto I. Evaluation of a mass screening program for stomach cancer with a case control study design. Int J Cancer 1986;38:829–33.

[3] Fukao A, Tsubono Y, Tsuji I, Hisamichi S, Sugahara N, Takano A. The evaluation of screening for gastric cancer in Miyagi Prefecture, Japan: a population-based case-control study. Int J Cancer 1995;60:45–8.

[4] Abe Y, Mitsushima T, Nagatani K, Ikuma H. Minamihira Y. Epidemiological evaluation of the protective effect for dying of stomach cancer by screening program for stomach cancer with applying a method of case-control study: a study of an efficient screening program for stomach cancer. J Jpn Soc Gastroenterol 1995;92:836–45 (in Japanese with English abstract).

[5] Lee KJ, Inoue M, Otani T, Iwasaki M, Sasazuki S, Tsugane S. Gastric cancer screening and subsequent risk of gastric cancer: a large-scale population-based cohort study, with a 13-year follow-up in Japan. Int J Cancer 2006;118:2315–21.

[6] Samloff IM, Varis K, Ihamaki T, Siurala M, Rotter JI. Relationships among serum pepsinogen I, serum pepsinogen II, and gastric mucosal histology. A study in relatives of patients with pernicious anemia. Gastroenterology 1982;83: 204–9.

[7] Miki K, Ichinose M, Shimizu A, Huang SC, Oka H, Furihata C, et al. Serum pepsinogens as a screening test of extensive chronic gastritis. Gastroenterol Jpn 1987;22:133–41.

[8] Kiyohira K, Yoshihara M, Ito M, Haruma K, Tanaka S, Chayama K. Serum pepsinogen concentration as a marker of *Helicobacter pylori* infection and the histologic grade of gastritis; evaluation of gastric mucosa by serum pepsinogen levels. J Gastroenterol 2003;38:332–8.

[9] Kawaguchi H, Haruma K, Komoto K, Yoshihara M, Sumii K, Kajiyama G. *Helicobacter pylori* infection is the major risk factor for atrophic gastritis. Am J Gastroenterol 1996;91: 959–62.

[10] Watanabe Y, Ozasa K, Higashi A, Hayashi K, Mizuno S, Mukai M, et al. *Helicobacter pylori* infection and atrophic gastritis. A case-control study in a rural town of Japan. J Clin Gastroenterol 1997;25:391–4.

[11] Correa P. *Helicobacter pylori* and gastric carcinogenesis. Am J Surg Pathol 1995;19(Suppl 1):S37–43.

[12] Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, et al. *Helicobacter pylori* infection and the development of gastric cancer. N Eng J Med 2001; 345:784–9.

[13] Hiyama T, Haruma K, Kitadai Y, Masuda H, Miyamoto M, Tanaka S, et al. K-*ras* mutation in *Helicobacter pylori*-associated chronic gastritis in patients with and without gastric cancer. Int J Cancer 2002;97:562–6.

[14] Hiyama T, Tanaka S, Shima H, Kose K, Kitadai Y, Ito M, et al. Somatic mutation of mitochondrial DNA in *Helicobacter pylori*-associated chronic gastritis in patients with and without gastric cancer. Int J Mol Med 2003;12:169–74.

[15] Yoshihara M, Sumii K, Haruma K, Kiyohira K, Hattori N, Kitadai Y, et al. Correlation of ratio of serum pepsinogen I and II with prevalence of gastric cancer and adenoma in Japanese subjects. Am J Gastroenterol 1998;93:1090–6.

[16] Miki K, Ichinose M, Ishikawa KB, Yahagi N, Matsushima M, Kakei N, et al. Clinical application of serum pepsinogen I and II levels for mass screening to detect gastric cancer. Jpn J Cancer Res 1993;84:1086–90.

[17] Hattori Y, Tashiro H, Kawamoto T, Kodama Y. Sensitivity and specificity of mass screening for gastric cancer using the measurement of serum pepsinogens. Jpn J Cancer Res 1995; 86:1210–5.

[18] Kodoi A, Yoshihara M, Sumii K, Haruma K, Kajiyama G. Serum pepsinogen in screening for gastric cancer. J Gastroenterol 1995;30:452–60.

[19] Yoshihara M, Sumii K, Haruma K, Kiyohira K, Hattori N, Tanaka S, et al. The usefulness of gastric mass screening using serum pepsinogen levels compared with photofluorography. Hiroshima J Med Sci 1997;46:81–6.

[20] Kitahara F, Kobayashi K, Sato T, Kojima Y, Araki T, Fujino MA. Accuracy of screening for gastric cancer using serum pepsinogen concentrations. Gut 1999;44:693–7.

[21] Miki K, Morita M, Sasajima M, Hoshino R, Kanda E, Urita Y. Usefulness of gastric cancer screening using the serum pepsinogen test method. Am J Gastroenterol 2003;98:735–9.

[22] Dinis-Ribeiro M, Yamaki G, Miki K, Costa-Pereica A, Matsukawa M, Kurihara M. Meta-analysis on the validity of pepsinogen test for gastric carcinoma, dysplasia or chronic atrophic gastritis screening. J Med Screen 2004;11:141–7.

[23] Ogawa H, Kato I, Tominaga S. Family history of cancer among cancer patients. Jpn J Cancer Res 1985;76:113–8.

[24] Nouraie M, Pietinen P, Kamangar F, Dawsey SM, Abnet CC, Albanes D, et al. Fruits, vegetables, and antioxidants and risk of gastric cancer among male smokers. Cancer Epidemiol Biomarkers Prev 2005;14:2087–92.

[25] Yoshihara M, Sumii K, Haruma K. Gastric mass survey with measurement of serum pepsinogen levels in a local area. Situation and practical method of the pepsinogen method in a local area. J Gastroenterol Mass Survey 1996;34:325–30 (in Japanese with English abstract).

[26] Xie XF, Ito M, Yoshihara M, Haruma K, Tanaka S, Chayama K. Serum pepsinogen levels in the Japanese population: prospective study of 9 years of follow up. Hepatogastroenterology. In press.

Clinicopathologic and genetic characteristics of gastric cancer in young male and female patients

SHOGO SASAO[1], TORU HIYAMA[4], SHINJI TANAKA[3], MASAHARU YOSHIHARA[4], WATARU YASUI[2] and KAZUAKI CHAYAMA[1]

[1]Department of Medicine and Molecular Science; Division of Frontier Medical Science, [2]Department of Molecular Pathology, Division of Molecular Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University; [3]Department of Endoscopy, Hiroshima University Hospital, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551; [4]Health Service Center, Hiroshima University, 1-7-1 Kagamiyama, Higashihiroshima 739-8521, Japan

Received December 6, 2005; Accepted February 9, 2006

Abstract. The pathways of gastric cancer in young patients (40 years of age or younger) have not yet been determined. We therefore examined clinicopathologically and genetically 68 gastric cancers in young patients and 66 tumors in older patients (41 years of age or older). Mutations in B-*raf* and K-*ras* were identified by PCR-SSCP following sequencing. Microsatellite instability (MSI) and *hMLH3* mutations were also examined. Histopathologically, diffuse-type gastric cancer and cancer in the whole of the stomach were found significantly more often in young patients than in older patients (21% vs. 2%, P=0.0006, and 77% vs. 32%, P<0.0001, respectively). Genetically, MSI and *hMLH3* mutations were found significantly more often in tumors in young patients than in tumors in older patients (15% vs. 4%, P=0.040, and 9% vs. 0%, P=0.036, respectively). Tumors in young female patients were found significantly less often in the lower-third of the stomach and showed a significantly greater frequency of MSI, compared to tumors in young male patients (33% vs. 9%, P=0.046, 5% vs. 30%, P=0.010, respectively). These results suggest that the pathways of gastric carcinogenesis differ between young patients and older patients, and that the pathways differ between the sexes in young patients.

Introduction

Gastric cancer has been decreasing in incidence over the last decade, but it is still the second most common cause of cancer-related death worldwide (1). It occurs most frequently in individuals 50-70 years of age. Gastric cancer results from a combination of environmental factors and accumulation of specific genetic alterations. Environmental factors, such as *Helicobacter pylori (H. pylori)* infection and a high-salt diet, and genetic factors play important roles in gastric carcinogenesis (2,3). Genetic alterations, such as activation of oncogenes K-*ras* and B-*raf* and inactivation of tumor suppressor gene *p53*, play important roles in the development of gastric cancers (4). Dysfunction of DNA mismatch repair genes, which leads to microsatellite instability (MSI), also plays a crucial role (5).

Gastric cancers occurring in young patients (40 years of age or younger) account for less than 5% of all gastric cancers (6,7). There have been several reports comparing clinicopathologic and biologic characteristics of young patients and older patients (more than 40 years of age) (8). Young patients with gastric cancer, in comparison to older patients, are thought to show a more aggressive clinical course and have a poorer prognosis. Thus, gastric cancers in young patients may have different genetic profiles from those in older patients. Although genetic characterization of gastric cancers has been the focus of several studies, few have addressed this issue specifically in young patients. The genetic pathways in young patients have not yet been determined. We therefore examined genetic alterations in gastric cancers to clarify differences in the disease between young patients and older patients.

Materials and methods

Study subjects were 134 patients with gastric cancer (68 young patients and 66 older patients) treated surgically at Hiroshima University Hospital or an affiliated hospital during the period 1990 through 2004. The young patients with gastric cancer were enrolled consecutively, and the older patients were enrolled randomly. For each patient, both cancerous and normal tissues were obtained at surgery.

Four-micrometer-thick sections were prepared from formalin-fixed, paraffin-embedded specimens. The sections were stained with hematoxylin and eosin (H&E) for histologic examination. Gastric cancers were classified as intestinal-

Correspondence to: Dr Shinji Tanaka, Department of Endoscopy, Hiroshima University Hospital, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan
E-mail: colon@hiroshima-u.ac.jp

Key words: gastric cancer, microsatellite instability, *hMLH3*, B-*raf*, K-*ras*

Table I. Primer sets used in the present study.

Gene	Sequence
K-*ras*	sense: 5'-TTGTTGGATCATATTCGTCC-3' antisense: 5'-TCAAAGAATGGTCCTGGACC-3'
B-*raf*	
exon 11	sense: 5'-AAACACTTGGTAGACGGGAC-3' antisense: 5'-ACTTGTCACAATGTCACCACATT-3'
exon 15	sense: 5'-CTTCATGAAGACCTCACAGT-3' antisense: 5'-GGCCAAAAATTTAATCAGTGGA-3'
hMLH3	
codon 583-585	sense: 5'-GCCTTTTGCAACAACATTATGG-3' antisense: 5'-GTGGAACATAATTTAACTCGCC-3'
codon 672-674	sense: 5'-AGACATCAAAGATTTAGCCAGC-3' antisense: 5'-CTGTAGGTTCATTCTCTAGCC-3'
BAT26	sense: 5'-TGACTACTTTTGACTTCAGCC-3' antisense: 5'-AACCATTCAACATTTTTAACCC-3'

type or diffuse-type as defined by Lauren (9). Depth of invasion was classified as to the mucosa or submucosa (early stage) or to the muscularis propria or deeper (advanced stage). The presence of lymph node metastasis was also examined. To analyze the relationship between tumor location and genetic alterations, the stomach was divided into three parts: the upper, middle, and lower parts. *H. pylori* infection was examined histologically with Giemsa staining. The presence of follicular gastritis, a type of *H. pylori*-associated gastritis characterized by the presence of prominent lymphoid follicles in the mucosal layer of the stomach (10), was also examined in the patients.

Ten-micrometer-thick tissue sections were placed on glass slides and stained with H&E. The tissue sections were then dehydrated in graded ethanol solutions and dried without a cover glass. Cancerous and normal tissues on the slides were scraped up with sterile needles, separately, by a microdissection technique. DNA was extracted from the tissues with 20 μl of extraction buffer [100 mM of Tris-HCl; 2 mM of ethylene diamine tetraacetic acid (EDTA), pH 8.0; 400 μl/ml of proteinase K] at 55°C overnight. The tubes were boiled for 7 min to inactivate the proteinase K, and then 2 μl of the extracts was used for each polymerase chain reaction (PCR) amplification.

Each tumor was evaluated for MSI by analysis of the mononucleotide repeat, BAT26. The microsatellite assay was performed as described elsewhere (11). The primer sets used in the present study are shown in Table I. Briefly, each 15 μl reaction mixture containing 10-20 ng of genomic DNA; 6.7 mM of Tris-HCl, (pH 8.8); 6.7 mM of EDTA; 6.7 mM of $MgCl_2$; 0.33 μM of primer labeled with (γ-^{32}P)dATP; 0.175 μM of unlabeled primer; 1.5 mM of each deoxynucleotide triphosphate; and 0.75 units of AmpliTaq Gold DNA polymerase (Perkin-Elmer, Branchburg, NJ) was amplified for 40 cycles as follows: denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and strand elongation at 72°C for 30 sec. The PCR products were electrophoresed on 6% polyacrylamide-8 M urea-32% formamide gels and autoradiographed overnight at -80°C with Fuji RX film. When additional bands appeared in the tumor DNA on the BAT26 marker, the tumor was defined as MSI-positive. Two mononucleotide repeats (poly A tracts) of *hMLH3* were also examined (12).

B-*raf* and K-*ras* genes were also examined. PCR-single-strand conformation polymorphism (SSCP) analysis was performed as described previously (4). The aberrant migration band on the SSCP gel was removed, amplified again, and directly sequenced on both strands with an ABI PRISM 310

Table II. Clinicopathologic characteristics of young and older patients with gastric cancer.

Characteristics	Young patients	Older patients	P-value
Sex			
Male/female	39/29	42/24	0.46
Tumor location			
Whole stomach/other/NA	11/42/15	1/65/0	0.0006
Histology			
Intestinal/diffuse	16/52	45/21	<0.0001
Tumor depth			
Early/advanced/NA	25/40/3	37/26/3	0.022
Helicobacter pylori infection			
Positive/negative	60/8	66/0	0.004
Follicular gastritis			
Present/absent	4/64	0/66	0.063

NA, information not available.

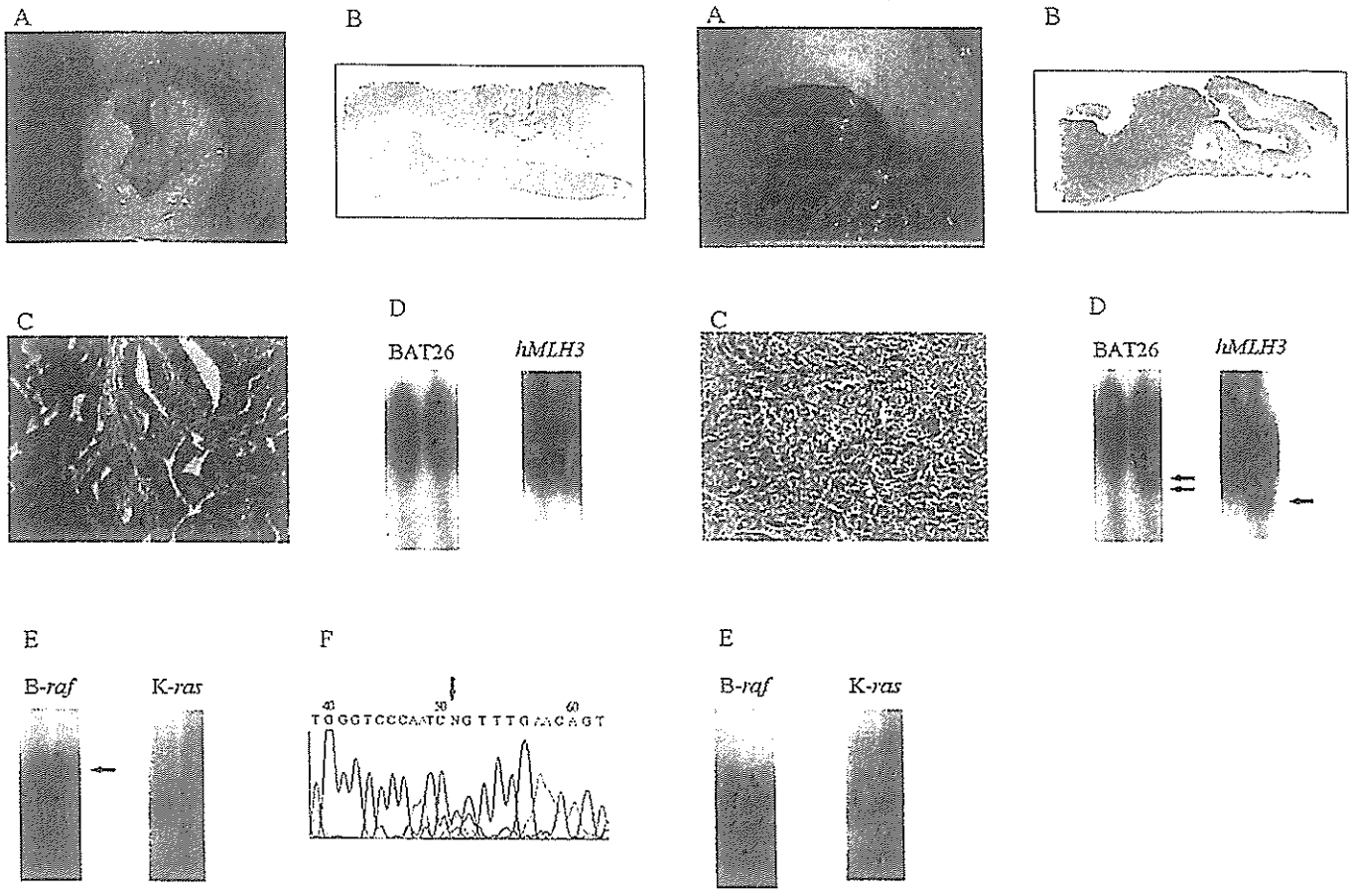

Figure 1. A representative case of intestinal-type gastric cancer. (A) Endoscopy revealed an ulcerated lesion at the greater curvature of the antral region. (B) Loupe appearance. (C) A histologically well-differentiated adenocarcinoma (x200) was identified. (D) Microsatellite analysis showed no alteration at BAT26 or *hMLH3*. (E) PCR-SSCP analysis showed mobility shift of B-*raf*. (F) Sequencing analysis of B-*raf* showed a CAG to CCG mutation at codon 608 of exon 15.

Figure 2. A representative case of diffuse-type gastric cancer. (A) Endoscopy revealed an ulcerated lesion at the posterior wall of the angular region. (B) Loupe appearance. (C) A histologically, diffuse, poorly differentiated adenocarcinoma (x200) was identified. (D) Microsatellite analysis showed alterations at BAT26 and *hMLH3*. (E) PCR-SSCP analysis showed no mobility shift of B-*raf* and K-*ras*.

genetic analyzer (Perkin-Elmer ABI, Foster City, CA). For the sequencing reaction, a PRISM AmpliTaq DNA polymerase FS ready reaction dye terminator sequencing kit (Perkin-Elmer ABI) was used.

Fisher's exact probability and Chi-square tests were used for comparisons of clinicopathologic and genetic factors. $P<0.05$ was regarded as significant.

Results

Representative cases are shown in Figs. 1 and 2. Clinicopathologic characteristics of young patients and older patients with gastric cancer are shown in Table II. The mean age of young gastric cancer patients was 35.3 (range, 18-40), and that of older gastric cancer patients was 64.5 (range, 44-90). Cancer in the whole of the stomach was found significantly more often in young patients than in older patients [11/53 (21%) vs. 1/66 (2%), respectively, P=0.0006 by Fisher's exact probability test]. Histopathologically, diffuse-type gastric cancer was found significantly more often in young patients than in older patients [52/68 (77%) vs. 21/66 (32%), respectively, P<0.0001 by Chi-square test]. Advanced tumors were significantly more prevalent in young patients than in older patients [40/65 (62%) vs. 26/63 (41%), respectively, P=0.022]. *H. pylori* infection was significantly less prevalent in young patients than in older patients [60/68 (88%) vs. 66/66 (100%), respectively, P=0.004]. Follicular gastritis was observed more frequently in young patients than in older patients [4/68 (6%) vs. 0/66 (0%), respectively, P=0.063 by Fisher's exact probability test].

Genetic characteristics of gastric cancers in young patients and in older patients are shown in Table III. MSI was found significantly more often in tumors in young patients than in tumors in older patients [10/65 (15%) vs. 2/51 (4%), respectively, P=0.040 by Fisher's exact probability test]. Mutations in *hMLH3* were found significantly more often in tumors in young patients than in tumors in older patients [5/56 (9%) vs. 0/51 (0%), respectively, P=0.036 by Fisher's exact probability test]. One detected K-*ras* mutation was a GGT to GCT (Gly to Ala) mutation at codon 12. Out of 10 B-*raf* mutations detected, 6 were CAT to CCG (His to Leu) mutations at codon 396 of exon 11, and 4 were CAG to CCG (Glu to Pro) mutations at codon 608 of exon 15. The frequencies of B-*raf* and K-*ras* mutations did not differ significantly between tumors in young patients and those in older patients.

The clinicopathologic characteristics of young gastric cancer patients are shown by sex in Table IV. There was a significant difference between the sexes in the number of tumors found in the lower-third of the stomach [2/22 females

Table III. Genetic characteristics of gastric cancer in young and older patients.

Genetic alteration	Young patients	Older patients	P-value
MSI			
+/-/NI	10/55/3 (15%)	2/49/15 (4%)	0.040
hMLH3 mutation			
+/-/NI	5/51/12 (9%)	0/51/15 (0%)	0.036
B-*raf* mutation			
+/-/NI	6/46/16 (12%)	4/46/16 (8%)	0.40
K-*ras* mutation			
+/-/NI	0/23/45 (0%)	1/32/33 (3%)	0.59

NI, not informative.

Table IV. Clinicopathologic characteristics of gastric cancer in young male and female patients.

Characteristics	Young male patients	Young female patients	P-value
Tumor location			
Lower third/others/NA	10/21/8	2/20/7	0.046
Histology			
Intestinal diffuse	11/28	5/24	0.22
Tumor depth			
Early/advanced/NA	15/21/3	10/19/0	0.55
Helicobacter pylori infection			
Positive/negative	34/5	26/3	0.53
Follicular gastritis			
Present/absent	2/37	2/27	0.57

NA, information not available.

(9%) vs. 10/31 males (33%), P=0.046 by Fisher's exact probability test].

The genetic characteristics of gastric cancer in young patients are shown by sex in Table V. MSI was found significantly more often in female patients than in male patients [8/27 (30%) vs. 2/38 (5%), respectively, P=0.010 by Fisher's exact probability test]. There were no significant differences in the frequencies of *hMLH3*, B-*raf*, and K-*ras* mutations between the sexes in young gastric cancer patients.

Table V. Genetic characteristics of gastric cancer in young male and female patients.

Genetic alteration	Male patients	Female patients	P-value
MSI			
+/-/NI	2/36/1 (5%)	8/19/2 (30%)	0.010
hMLH3 mutation			
+/-/NI	2/30/7 (6%)	3/21/5 (13%)	0.36
B-*raf* mutation			
+/-/NI	4/28/7 (13%)	2/18/9 (10%)	0.58
K-*ras* mutation			
+/-/NI	0/13/26 (0%)	0/10/19 (0%)	-

NI, not informative.

Discussion

Gastric cancers in young patients are reported to have different clinicopathologic characteristics from gastric cancers in older patients. For instance, diffuse-type gastric tumors and liver metastasis are reported to occur significantly more frequently in young patients than in older patients, and young patients are reported to have a more aggressive phenotype and poorer prognosis than older patients (8). These findings suggest that the pathways of gastric cancer in young patients differ from those in older patients. The increased frequency of cancer of the whole stomach and diffuse-type gastric cancer that we observed in young patients is similar to results reported previously. These data also suggest that the pathways of gastric cancer in young patients may differ from those in older patients.

We examined genetic alterations in gastric cancer to clarify whether the molecular profiles of tumors differ between young patients and older patients. Gastric cancer can occur as a hereditary non-polyposis colorectal cancer, whereby alterations in the mismatch repair genes (*hMLH1*, *hMSH2*, *hMSH6*, etc.) are responsible for colorectal, gastric, and endometrial tumor formation (13). Disrupted function of mismatch repair genes manifests as MSI and has been reported in 15-39% of sporadic gastric cancer. A single test of BAT26 can identify cases positive for high-level MSI (14,15). Several researchers have reported that MSI is rare (0-1.3%) in gastric cancer in young patients. However, Hayden *et al* (6) reported MSI in 6% of gastric cancers in young patients, and Semba *et al* (16) reported MSI in 22% of gastric cancers in young patients. In the present study, 15% of gastric cancers in young patients showed MSI, and MSI was found significantly more often in tumors in young patients than in tumors in older patients. In addition, tumors in young patients had significantly frequent mutations of *hMLH3*, one of the mismatch repair genes, compared with tumors in older patients. Mutation of the major mismatch repair genes, *hMSH2* and *hMLH1*, has not been detected in gastric cancers in young patients (17). Thus, it is possible that *hMLH3* mutation is a key genetic change in the development of gastric cancer in young patients. The number of cases examined in the present study was limited; further examination of genetic changes in a greater number of cases may be necessary.

We previously reported that follicular gastritis confers a high-risk for diffuse-type gastric cancer and is predominant in female patients (10,18). In the present study, cancer in the whole of the stomach and MSI were was found significantly more often in young females than in young males, and follicular gastritis tended to be more common in young females than in young males. These results suggest differences between the sexes in the genetic pathways of gastric cancer in young patients. This is the first reported study to focus on differences between the sexes regarding the clinicopathologic and genetic characteristics of gastric cancer in young patients.

ras genes are the most frequently mutated oncogenes in human cancers (19). The vast majority of *ras* mutations associated with human diseases involve K-*ras* (20). Activating point mutations of the gene affect codons 12 and 13. K-*ras* mutations are reported in 2.8-20% of gastric cancers (4). In the present study, K-*ras* mutations were detected in only 3% of tumors in older patients and in none of the tumors in young patients. The results were similar to those reported previously.

Recently, B-*raf* mutations have been reported in human malignancies, such as colon cancer and melanoma (21). Almost all reported B-*raf* mutations have occurred within two kinase domains (the G-loop domain and kinase domain), and the most common mutation is a single substitution, V599E. B-*raf* protein plays a central role in the ras/raf/mek/erk pathway, relaying signals from activated RAS proteins. B-*raf* mutations in gastric cancer are reportedly infrequent at 0-2.2% (22,23). In the present study, B-*raf* mutations were detected in 12% of tumors in young patients and in 8% of tumors in older patients. The percentages in the present study were high compared to those reported previously. However, there was no significant difference in the frequency of B-*raf* mutations between young patients and older patients.

The B-*raf* and K-*ras* mutations were infrequent, and there were no significant differences in the frequencies of these gene mutations between the sexes. These genes may not be key genetic alterations in the development of gastric cancers.

In conclusion, the clinicopathologic and genetic differences in gastric cancer that we observed between young patients and older patients suggest that the pathways of gastric cancer development differ between these two groups. In addition, the clinicopathologic and genetic differences that we observed between gastric cancer in young male patients and that in young female patients suggest different pathways. Thus, even among young patients, the pathways of gastric cancer development may differ, depending on sex.

References

1. Parkin DM, Pisani P and Ferlay J: Estimates of the worldwide incidence of eighteen major cancers in 1985. Int J Cancer 54: 594-606, 1993.
2. Meyers WC, Damiano RJ Jr, Rotolo FS and Postlethwait RW: Adenocarcinoma of the stomach. Changing patterns over the last 4 decades. Ann Surg 205: 1-8, 1987.
3. Miyoshi E, Haruma K, Hiyama T, Tanaka S, Yoshihara M, Shimamoto F and Chayama K: Microsatellite instability is a genetic marker for the development of multiple gastric cancers. Int J Cancer 95: 350-353, 2001.
4. Hiyama T, Haruma K, Kitadai Y, Masuda H, Miyamoto M, Tanaka S, Yoshihara M, Shimamoto F and Chayama K: K-*ras* mutation in *Helicobacter pylori*-associated chronic gastritis in patients with and without gastric cancer. Int J Cancer 97: 562-566, 2002.
5. Hiyama T, Tanaka S, Yoshihara M, Sasao S, Kose K, Shima H, Tuncel H, Ueno Y, Ito M, Kitadai Y, Yasui W, Haruma K and Chayama K: Chromosomal and microsatellite instability in sporadic gastric cancer. J Gastroenterol Hepatol 19: 756-760, 2004.
6. Hayden JD, Cawkwell L, Sue-Ling H, Johnston D, Dixon MF, Quirke P and Martin IG: Assessment of microsatellite alterations in young patients with gastric adenocarcinoma. Cancer 79: 684-687, 1997.
7. Mori M, Sugimachi K, Ohiwa T, Okamura T, Tamura S and Inokuchi K: Early gastric carcinoma in Japanese patients under 30 years of age. Br J Surg 72: 289-291, 1985.
8. Nakamura T, Yao T, Niho Y and Tsuneyoshi M: A clinicopathological study in young patients with gastric carcinoma. J Surg Oncol 71: 214-219, 1999.
9. Lauren P: The two histological main types of gastric carcinoma: Diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. APMIS 64: 31-49, 1965.
10. Miyamoto M, Haruma K, Hiyama T, Kamada T, Masuda H, Shimamoto F, Inoue K and Chayama K: High incidence of B-cell monoclonality in follicular gastritis: a possible association between follicular gastritis and MALT lymphoma. Virchows Arch 440: 376-380, 2002.
11. Hiyama T, Tanaka S, Kitadai Y, Ito M, Sumii M, Yoshihara M, Shimamoto F, Haruma K and Chayama K: *p53* codon 72 polymorphism in gastric cancer susceptibility in patients with *Helicobacter pylori*-associated chronic gastritis. Int J Cancer 100: 304-308, 2002.
12. Akiyama Y, Nagasaki H, Nakajima T, Sakai H, Nomizu T and Yuasa Y: Infrequent frameshift mutations in the simple repeat sequences of *hMLH3* in hereditary nonpolyposis colorectal cancers. Jpn J Clin Oncol 31: 61-64, 2001.
13. Wu M, Semba S, Oue N, Ikehara N, Yasui W and Yokozaki H: BRAF/K-ras mutation, microsatellite instability, and promoter hypermethylation of hMLH1/MGMT in human gastric carcinomas. Gastric Cancer 7: 246-253, 2004.
14. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN and Srivastava S: A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 58: 5248-5257, 1998.
15. Hoang JM, Cottu PH, Thuille B, Salmon RJ, Thomas G and Hamelin R: BAT-26, an indicator of the replication error phenotype in colorectal cancers and cell lines. Cancer Res 57: 300-303, 1997.
16. Semba S, Yokozaki H, Yasui W and Tahara E: Frequent microsatellite instability and loss of heterozygosity in the region including BRCA1 (17q21) in young patients with gastric cancer. Int J Oncol 12: 1245-1251, 1998.
17. Lim S, Lee HS, Kim HS, Kim YI and Kim WH: Alteration of E-cadherin-mediated adhesion protein is common, but microsatellite instability is uncommon in young age gastric cancers. Histopathology 42: 128-136, 2003.
18. Miyamoto M, Haruma K, Yoshihara M, Sumioka M, Nishisaka T, Tanaka S, Inoue K and Chayama K: Five cases of nodular gastritis and gastric cancer : a possible association between nodular gastritis and gastric cancer. Dig Liver Dis 34: 819-820, 2002.
19. Kiaris H and Spandidos DA: Mutations of *ras* genes in human tumours (Review). Int J Oncol 7: 413-421, 1995.
20. Ellis CA and Clark G: The importance of being K-Ras. Cell Signal 12: 425-434, 2000.
21. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Hawes R, Hughes J, Kosmidou V, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chenevix-Trench G, Riggins GJ, Bigner DD, Palmieri G, Cossu A, Flanagan A, Nicholson A, Ho JW, Leung SY, Yuen ST, Weber BL, Seigler HF, Darrow TL, Paterson H, Marais R, Marshall CJ, Wooster R, Stratton MR and Futreal PA: Mutations of the BRAF gene in human cancer. Nature 417: 949-954, 2002.
22. Lee SH, Lee JW, Soung YH, Kim HS, Park WS, Kim SY, Lee JH, Park JY, Cho YG, Kim CJ, Nam SW, Kim SH, Lee JY and Yoo NJ: BRAF and KRAS mutations in stomach cancer. Oncogene 22: 6942-6945, 2003.
23. Oliveira C, Pinto M, Duval A, Brennetot C, Domingo E, Espin E, Armengol M, Hamelin R, Seruca R and Schwartz S Jr: BRAF mutations characterize colon but not gastric cancer with mismatch repair deficiency. Oncogene 22: 9192-9196, 2003.

Original Paper

Digestion

Digestion 2006;73:47–53
DOI: 10.1159/000092014

Received: November 15, 2005
Accepted: January 5, 2006
Published online: March 14, 2006

Development of a Novel Method to Detect *Helicobacter pylori cagA* Genotype from Paraffin-Embedded Materials: Comparison between Patients with Duodenal Ulcer and Gastric Cancer in Young Japanese

Hiroyuki Ueda[a] Masanori Ito[a] Hidetaka Eguchi[b] Shinji Tanaka[c]
Masaharu Yoshihara[d] Ken Haruma[e] Masanori Hatakeyama[f]
Kazuaki Chayama[a]

[a]Department of Medicine and Molecular Science, Hiroshima University, [b]Department of Radiobiology/Molecular Epidemiology, Radiation Effects Research Foundation, [c]Department of Endoscopy, Hiroshima University Hospital, Hiroshima; [d]Health Service Center, Hiroshima University, Higashi-Hiroshima; [e]Gastroenterology Unit, Department of Internal Medicine, Kawasaki Medical School, Kurashiki, and [f]Division of Molecular Oncology, Institute for Genetic Medicine, Graduate School of Science, Hokkaido University, Sapporo, Japan

Key Words

Helicobacter pylori · *cagA* gene · Gastric cancer · Duodenal ulcer · Paraffin-embedded section

Abstract

Background/Aim: *cagA* gene polymorphism of *Helicobacter pylori* contributes to clinical outcome of patients. We investigated the implication of the *cagA* polymorphism in young Japanese patients using paraffin-embedded sections. ***Methods:*** We studied 71 young patients with gastric cancer or with duodenal ulcer. *H. pylori* infection was confirmed by sections with Giemsa staining and immunohistochemical staining and the degree of gastritis was evaluated. DNA was extracted from paraffin-embedded sections of 20 patients both from the gastric corpus and the antrum. A portion of *cagA* gene was amplified with polymerase chain reaction, followed by direct sequencing of the fragment. ***Results:*** We established a novel method to determine the *cagA* subtype using paraffin-embedded sections. We found that all the samples possessed East-Asian type *cagA* both in the corpus and the antrum, not only in patients with gastric cancer but also with duodenal ulcer. Although the *cagA* gene sequence was completely identical between the gastric corpus and the antrum in all patients, the corpus gastritis was more prominent in patients with gastric cancer than those with duodenal ulcer. ***Conclusions:*** *cagA* polymorphism can be evaluated with the use of paraffin-embedded sections. The degree of corpus gastritis may not be regulated by *cagA* diversity only.

Introduction

Helicobacter pylori infection is a critical factor for promoting atrophic gastritis [1]. Long-term infection of *H. pylori* results in glandular atrophy and intestinal metaplasia. Since corpus atrophic gastritis is a fundamental and essential status for human gastric carcinogenesis, *H. pylori* is regarded as an important carcinogen in the devel-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0012–2823/06/0731–0047$23.50/0

Accessible online at:
www.karger.com/dig

Masanori Ito, MD, PhD
Department of Medicine and Molecular Science
Hiroshima University, Hiroshima 734-8551 (Japan)
Tel. +81 82 257 5191, Fax +81 82 257 5194
E-Mail maito@hiroshima-u.ac.jp

opment of human gastric cancer [2]. Indeed, it has been accepted that there is a strong association between *H. pylori*-associated gastritis and gastric cancer [3–6]. A clinical study by Uemura et al. [8] confirmed a strong association between the occurrence of gastric cancer and *H. pylori* infection followed by corpus gastritis. Atrophic gastritis and gastric cancer are very common diseases in Japan, since the high prevalence of *H. pylori* infection has especially been confirmed in elderly people [9]. However, not all patients with *H. pylori* infection have gastric cancer, so it is clinically important to select the population with a high risk for gastric cancer development [10].

H. pylori cagA protein is an important virulent factor for gastric mucosa injury. Huang et al. [11] demonstrated the strong association between anti-cagA seropositivity and development of gastric cancer, suggesting an importance of cagA for gastric carcinogenesis. Recent studies have clarified the molecular mechanism of gastric mucosal injury induced by cagA protein. cagA protein produced in the bacterial cell is translocated into the host cell by the type IV secretory system, followed by tyrosine phosphorylation by src-family kinases and activation of SHP-2 phosphatase [12, 13]. Recent studies revealed that cagA protein showed diversity and was subclassified into two types, namely Western type and East-Asian type. The latter type was reported to have a high affinity to SHP-2 and was thus regarded as a more harmful form than the Western type [14]. In fact, diversity of cagA protein was assessed and showed a tight relationship between its diversity and the clinical outcome [15].

However, until now, most studies have been performed with the use of isolated colonies, resulting in the uncertainty of whether it really reflects the original character of *H. pylori* in the stomach. In addition, it is clinically important to investigate the topography of gastric inflammation, which may be influenced by the heterogeneous distribution of *H. pylori*. Then, in the present study, we investigated the cagA subtype of *H. pylori* with the use of DNA samples extracted from paraffin-embedded sections that reflect the original character of the bacteria. Moreover, we studied the diversity in the samples from the gastric corpus and the antrum separately to discuss the heterogeneity of the cagA subtype in the stomach.

Methods

Patients

We studied 32 patients with gastric cancer (20 men, mean age 26.6 years; 12 women, mean age 25.6 years) and 39 patients with duodenal ulcer (36 men, mean age 25.1 years; 3 women, mean age 25.6 years). All patients were <30 years and underwent gastrectomy. The resected stomach was fixed in buffered formalin and embedded in a paraffin block. Except for the pathological lesions, non-neoplastic gastric mucosae in the lesser curvature were cut and embedded in paraffin sections in the same manner. These non-neoplastic mucosas were used for the present examinations. All patients had histological gastritis either in the gastric corpus and antrum, and were confirmed as being *H. pylori*-positive by Giemsa staining. Patients who received eradication therapy were not included in this study. We obtained informed consent from all patients and the Ethical Committee of Hiroshima University approved our protocol.

Evaluation of Histology of Gastritis

In each patient, histological gastritis of the lesser curvature of the corpus and antrum were evaluated with the use of the sections stained with hematoxylin and eosin (HE). We scored the degree of gastritis (mononuclear infiltration and activity; from 0 to 3) with the criteria of the updated Sydney System [16]. Two specialists (M.I. and K.H.) who independently scored the degree of gastritis were blind to the clinical information.

DNA Extraction from Paraffin-Embedded Sections

We randomly selected 20 patients (10 with gastric cancer and 10 with duodenal ulcer) and conducted DNA extraction from the tissue sections of the patients. Tissue sections of 4 μm in thickness were placed on glass slides and stained with HE. The tissue sections were then dehydrated in graded ethanol solutions and dried without a cover glass. Tissues were scraped from the slides with sterile needles. DNA was extracted from the tissues by incubation in 20 μl of extraction buffer (100 m*M* Tris-HCl; 2 m*M* EDTA, pH 8.0; 400 μg/μl proteinase K) at 55°C overnight. The tubes were boiled for 7 min to inactivate the proteinase K, and then 2 μl of the extracts was used for each polymerase chain reaction (PCR) amplification.

PCR Reaction

To determine the cagA subtype, two pairs of primers (forward: cagA01 and reverse: cagA02 or cagA05, respectively) were used for PCR amplification, yielding 117- and 92-basepair (bp) products, respectively (table 1). Each 20 μl of reaction mixture contained 0.2 μl of Pyrobest DNA polymerase (5 units/μl; Takara, Shiga, Japan), 2 μl of 10× Pyrobest buffer II, 2 μl of dNTP mixture (2.5 m*M* each), and 1 μl each of forward and reverse primers. The reaction mixtures were heated to 95°C for 5 min, followed by 50 cycles of denaturation at 95°C for 30 s, annealing at 63°C for 30 s, and elongation at 72°C for 30 s. After PCR, the products were electrophoresed on 8% polyacrylamide gels containing 1× TBE buffer (50 m*M* Trizma base, 67 m*M* borate, 1 m*M* EDTA).

Sequencing

DNA bands were excised from the gels and the DNAs were eluted and purified using QIAquick Gel Extraction kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. Then, purified DNA fragments were subjected to sequence reaction using BigDye Terminators Version 1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, Calif., USA) and ABI Prism 310 Genetic Analyzer (Applied Biosystems) according to the manufacturer's instruction.

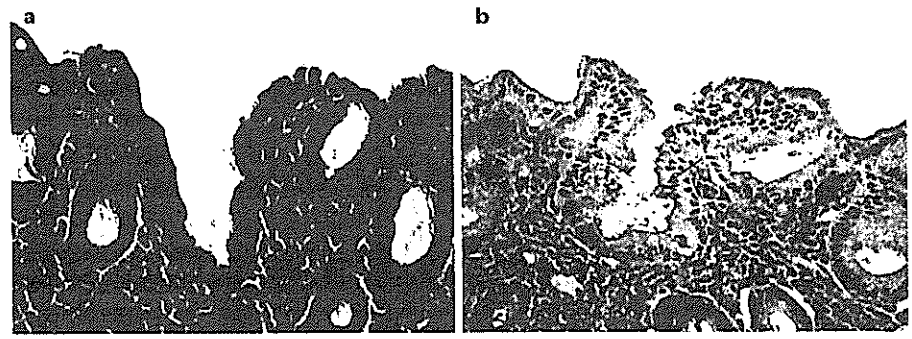

Fig. 1. Detection of *H. pylori* in the gastric mucosa from the corpus by HE section (**a**) and by immunohistochemical staining (**b**) in a 26-year-old male patient. Arrow indicates the bacterium just on the epithelial cells.

Table 1. Histology in the corpus and antrum of the patient groups

	Corpus			Antrum		
	GC (n = 30)	DU (n = 36)	p	GC (n = 32)	DU (n = 39)	p
Infiltration						
Grade 0 or 1, n (%)	10 (33.3)	32 (88.9)		8 (25.0)	6 (15.4)	
Grade 2 or 3, n (%)	20 (66.7)	4 (11.1)	<0.001	24 (75.0)	33 (84.6)	>0.3
Activity						
Grade 0 or 1, n (%)	20 (66.7)	35 (97.2)		19 (59.4)	20 (51.3)	
Grade 2 or 3, n (%)	10 (33.3)	1 (2.8)	0.002	13 (40.6)	19 (48.7)	>0.4

Immunohistochemistry

Four-micrometer sections of formalin-fixed paraffin-embedded tissues were used for immunohistochemical staining. After deparaffinization and hydration, internal peroxidase was blocked by incubation with 0.3% H_2O_2 in methanol for 15 min. After incubation with 5% skim milk/PBS for 20 min, the sections were reacted with the primary antibody (diluted with PBS) for 2 h at room temperature. The primary antibody used was anti-*H. pylori* polyclonal antibody (dilution of 1:50; Dako, Kyoto, Japan). Antigen retrieval was carried out with microwave treatment before reacting with primary antibody.

Statistics

Statistical analysis was performed by χ^2 test and Fisher's exact test with SPSS Version 11.5J software (SPSS Inc., Chicago, Ill., USA). A p value of <0.05 was considered statistically significant.

Results

Immunohistochemical Detection of H. pylori

We examined the *H. pylori* status by immunohistochemical staining. As shown in figure 1, we could hardly detect the bacteria in HE sections. In most sections the mucous layer over the mucosal epithelium had been washed out and could not be found in the sections. In immunohistochemical analysis, several bacteria could be detected just on the epithelium. The image is not different between sections from the two groups (gastric cancer and duodenal ulcer).

Comparison of Histology in Gastritis between Gastric Cancer and Duodenal Ulcer Patients

First, we compared the grades of gastritis between sections from young patients with gastric cancer and with duodenal ulcer. As shown in table 1, the degree of antral gastritis was not statistically different in neutrophil activity and chronic inflammation between these two groups. On the other hand, in the gastric corpus the degree of gastritis was statistically more prominent (activity, $p = 0.002$, and chronic inflammation, $p < 0.001$) in sections with gastric cancer than in those with duodenal ulcer.

Establishment of Amplification Method of cagA *Gene Using Paraffin-Embedded Sections*

Since DNA samples were degraded in various degrees and the amount of *H. pylori* DNA relative to the human DNA was small, many experimental improvements

cagA Analysis from Paraffin-Embedded Section

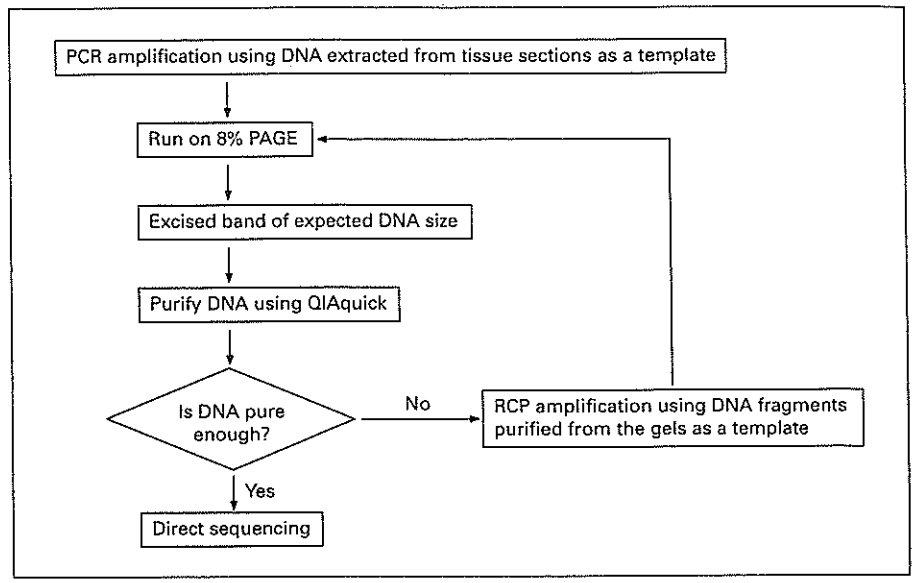

Fig. 2. The method from PCR amplification to direct sequence.

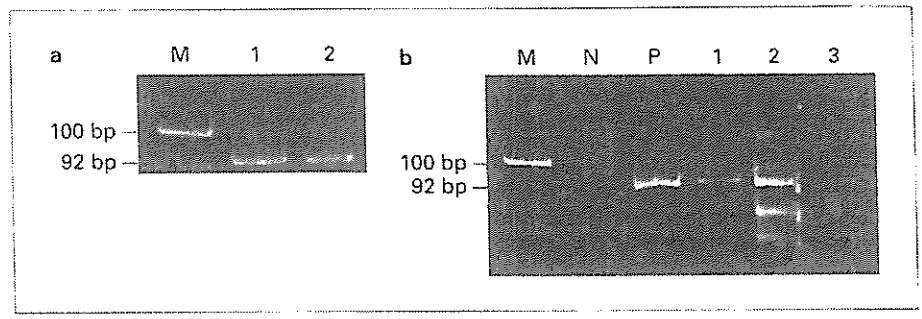

Fig. 3. Detection of PCR products. **a** Products with single amplification (lane 1) and those by repeated PCR three times (lane 2). **b** Final products of amplified *H. pylori* DNA from 3 patients (lanes 1–3). M = Marker; N = negative control; P = positive control.

Table 2. Oligonucleotides used to detect the CagA

Gene	Primer	Primer sequence	Size, bp
cagA	cagA01	5′-TAGCCCTGAACCCATTTACG-3′	(01-02) 117
	cagA02	5′-TGTTCCCTTGAAAGCCCTAC-3′	
	cagA05	5′-TGAGATCACTAACTGCAGCAC-3′	(01-05) 92

were needed to obtain PCR products of good quality capable for conducting direct sequence reaction (fig. 2). First, we found that PCR products should not exceed 100 bp in size to obtain reproducible amplification. Primers that were used in this study are shown in table 2. For the PCR amplification, Pyrobest DNA polymerase possessing proofreading activity was suitable for our experiments. Since the first PCR products contained additional DNA fragments (fig. 3a, lane 1), the amplified fragments were separated and purified from the 8% PAGE and the purified DNA fragments were used as a template for second or third PCR to obtain a single band of *cagA* fragment (fig. 3a, lane 2). In order to confirm that obtained PCR fragments were specific for *cagA*, we always included negative controls in each reaction (fig. 3b). As for the reproducibility of this method, we confirmed that we could obtain completely identical results by repeating the examination with the same section (data not shown).

Comparison of cagA Subtype between Gastric Cancer and Duodenal Ulcer Patients

Using direct sequencing, we were able to obtain the *cagA* DNA sequences from all the tested samples. The

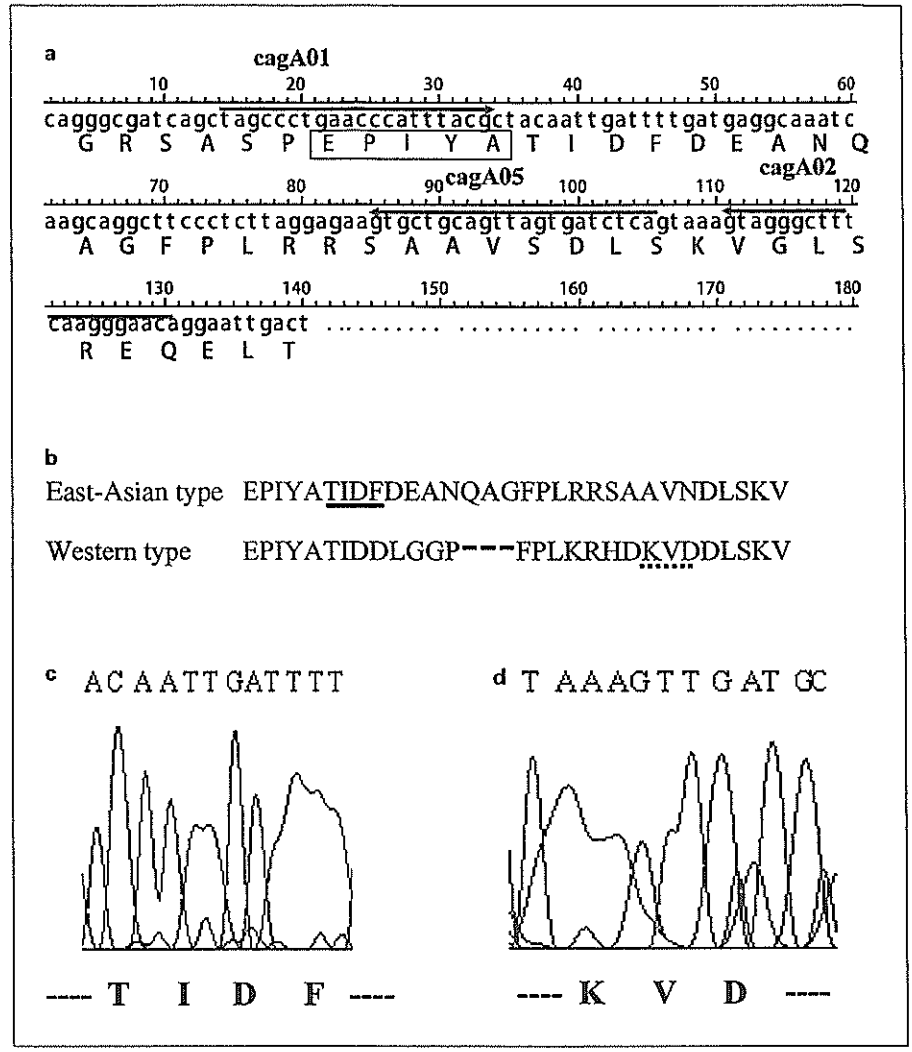

Fig. 4. a The information about the place of primers (cagA 01, 02 and 05). **b** Representative amino acid sequence of East-Asian and Western type cagA protein [from 14]. Results of direct sequence of DNA extracted from paraffin-embedded section with the use of primers cagA01-02 (**c**, **d**). The results of direct sequence of *cag*A DNA (upper) and encoding amino acids cagA protein (lower) were demonstrated. **c** The sequence of East-Asian cagA (#974–977, +2 to +5 from Y; black line in **b**). **d** The sequence of Western cagA (+17 to +19 from Y; dotted line in **b**). The patient used in figure 4d was not included in our study.

information about the place of primers used in our study is demonstrated in figure 4a, and typical amino acid sequences of two cagA subtypes are also demonstrated in figure 4b. The representative results are shown in figure 4c. In gastric cancer patients, we detected only East-Asian type cagA both in the gastric corpus and antrum. In duodenal ulcer patients, the same results were obtained not only in the gastric corpus but also in the antrum. Although we detected only East-Asian type cagA in the present study, we confirmed that the Western type cagA could be detected in another examination and denied the possibility that our system could only be applicable for detecting East-Asian type cagA (fig. 4d).

cagA Subtype Heterogeneity between the Gastric Antrum and the Corpus

Then, we compared all the DNA sequences encoding amino acids of cagA protein between those from the gastric corpus and from the antrum. The DNA sequence was completely identical between the DNAs extracted from the different sites.

Discussion

East-Asian cagA is supposed to be more virulent to epithelial injury and is regarded as being more carcinogenic for gastric mucosa than the Western type. In fact, the international distribution of the gastric cancer incidence could be explained by the diversity of this harmful

type of cagA [17]. However, in Japanese patients, almost all bacteria had cagA protein and have revealed that its subtype was the East-Asian type [18, 19]. Therefore, it is difficult to explain the difference in clinical outcome induced by *H. pylori* infection within Japanese patients only by the diversity of cagA status.

Focus must be placed on the methodology of these studies. In most studies, samples were extracted from isolated colonies of *H. pylori*. In the status, it is controversial whether the biological characters of isolated colonies reflect the original character of the bacteria in vivo, since the presence of metastability of the *H. pylori* could not be completely denied [20]. Another important problem is the topography of gastric inflammation. In gastric carcinogenesis, corpus-predominant gastritis showed a tighter relationship with gastric cancer, whereas antral-predominant gastritis is a negative factor for gastric carcinogenesis [8]. It is difficult to explain the heterogeneous distribution of gastritis inflammation only from the difference of solitary bacteria.

In the present study, we examined and compared the difference in the status of gastritis between two groups: the first included gastric cancer patients <30 years whose *H. pylori* was expected to be virulent in gastric carcinogenesis and the other included duodenal ulcer patients who were considered to have *H. pylori* that is less potential in gastric carcinogenesis. We have previously reported the tight association between *H. pylori* infection and the occurrence of gastric cancer in young patients [21, 22]. We then compared the status of *H. pylori* cagA status in these two groups, and the improved points in this study were (1) to use the paraffin-embedded section to avoid the metastability of the bacteria, and (2) to examine the status of gastric corpus and antrum separately in each patient.

In the present study, we could confirm that corpus gastritis was more prominent in gastric cancer patients than in duodenal ulcer patients, whereas antral gastritis was not different between these two groups. These findings are completely compatible with the report by Uemura et al. [8]. However, unexpectedly, the cagA status was not different between these two groups not only in the gastric antrum but also in the gastric corpus. This suggests that the status of gastritis, especially in the gastric corpus, is not affected by the status of the cagA protein in Japanese subjects. The main cause is still unclear but this might be regulated in the step of bacterial adhesion or of intracellular signaling after SHP-2 activation. A previous report has demonstrated that intragastric status including acid secretion may be a key factor for the mechanism of corpus inflammation induced by the cagA infusion system [23], but some controversial results have also been published. It is still controversial whether *H. pylori* in the stomach is monotonous or heterogeneous. We previously studied the bacterial resistance for antibiotics and demonstrated that the heterogeneity of *H. pylori* could be found in approximately 30% of the patients [24]. In the present study, the sequence of the cagA hot spot is completely identical between gastric corpus and the antrum in all cases examined. It is unlikely that the heterogeneous distribution as for cagA subtype could be detected in Japanese patients. Differing from the drug resistance gene, *cagA* gene may be highly conserved and not be under the status of easy metastability.

The main advantage in this study is the establishment of a methodology to examine the cagA status using paraffin-embedded sections. Fortunately, by using the paraffin-embedded surgical sections, we succeeded in reducing the influence of *H. pylori* floating in the gastric mucous. Previously, only cagA status (positive or negative) was determined by using paraffin-embedded sections, and no report could be found as for the direct sequencing of *cagA* gene [25, 26]. Our method seems to be time- and money-consuming comparing the analysis with the use of fresh biopsy specimens. However, in old and rare cases, such as gastric carcinoma in young patients, often we only have to use the paraffin-embedded sections as a starting material. This is the main purpose of our study and we believe our method may contribute to the further extension in this field including retrospective studies using old samples.

However, our protocol may be laborious since it requires the repeated PCR reactions to obtain a high-quality result. For example, as in our experience, the size of the PCR product was limited to 100 bp in length and three times repeated PCR was needed for most cases. In addition, our experience showed that the results were affected by the conditions of sample preparation including the fixation procedure. An immediate fixation after resection may be important for the maintenance of the good quality of *H. pylori* DNA, and the use of buffered formalin seemed to be essential. Long-term washing of fixed samples may result in extreme reduction of bacterial number and should be avoided. Further technical improvement of our method may be required for application to the clinical examination in practice. In the next step, it may be possible to distinguish two types of *cagA* gene without sequencing. Actually, the size of the PCR product in each subtype was different (117 vs. 108 bp) when we used cagA 01-02 primers. As demonstrated in figure 3, we found

some additional bands and these made it difficult to distinguish the cagA pattern only by evaluating the pattern after electrophoresis. However, it is important to try to make better primers for this purpose.

Taken together, this is the first report demonstrating the cagA status of gastric cancer and duodenal ulcer in young patients using paraffin-embedded sections. Our results will provide the next strategy to clarify the difference in corpus gastritis between these patients. The clarification of bacterial adhesion including the intragastric condition may be a next step in this matter.

Acknowledgements

The authors thank Dr. Kei Nakachi (Radiation Effects Research Foundation, Hiroshima, Japan) and Drs. Kenichi Imagawa and Yosuke Harada (Theranostics Research Center Otsuka Pharm. Co. Ltd, Tokushima, Japan) for the invaluable advice for our experiments. Also, we thank Dr. Chiaki Inokuchi (Inokuchi Hospital Higashi-Hiroshima, Japan) for her kind supply of surgically resected specimens.

References

1 Kawaguchi H, Haruma K, Komoto K, Yoshihara M, Sumii K, Kajiyama G: *Helicobacter pylori* infection is the major risk factor for atrophic gastritis. Am J Gastroenterol 1996;91:959–962.

2 Correa P: *Helicobacter pylori* and gastric carcinogenesis. Am J Surg Pathol 1995;19(suppl 1):S37–S43.

3 Watanabe T, Tada M, Nagai H, Sasaki S, Nakao M: *Helicobacter pylori* infection induces gastric cancer in Mongolian gerbils. Gastroenterology 1998;115:642–648.

4 Sugiyama A, Maruta F, Ikeno T, Ishida K, Kawasaki S, Katsuyama T, Shimizu N, Tatematsu M: *Helicobacter pylori* infection enhances *N*-methyl-*N*-nitrosourea-induced stomach carcinogenesis in the Mongolian gerbil. Cancer Res 1998;58:2067–2069.

5 Huang JQ, Sridhar S, Chen Y, Hunt RH: Meta-analysis of the relationship between *Helicobacter pylori* seropositivity and gastric cancer. Gastroenterology 1998;114:1169–1179.

6 Komoto K, Haruma K, Kamada T, Tanaka S, Yoshihara M, Sumii K, Kajiyama G: *Helicobacter pylori* infection and gastric neoplasia: correlations with histological gastritis and tumor histology. Am J Gastroenterol 1998;93:1271–1276.

7 International Agency for Research on Cancer Working Group on the Evaluation of Carcinogenic Risks to Humans. Schistosomes, Liver Flukes, and *Helicobacter pylori:* Lyon, France. International Agency for Research on Cancer; 1994:177–240.

8 Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ: *Helicobacter pylori* infection and the development of gastric cancer. N Engl J Med 2001;345:784–789.

9 Asaka M, Kimura T, Kudo M, Takeda H, Mitani S, Miyazaki T, Miki K, Graham DY: Relationship of *Helicobacter pylori* to serum pepsinogens in an asymptomatic Japanese population. Gastroenterology 1992;102:760–766.

10 Ito M, Tanaka S, Kamada T, Haruma K, Chayama K: Causal role of *Helicobacter pylori* infection and eradication therapy in gastric carcinogenesis. World J Gastroenterol 2006 (in press).

11 Huang JQ, Zheng GF, Sumanac K, Irvine EJ, Hunt RH: Meta-analysis of the relationship between cagA seropositivity and gastric cancer. Gastroenterology 2003;125:1636–1644.

12 Asahi M, Azuma T, Ito S, Ito Y, Suto H, Nagai Y, Tsubokawa M, Tohyama Y, Maeda S, Omata M, Suzuki T, Sasakawa C: *Helicobacter pylori* cagA protein can be tyrosine phosphorylated in gastric epithelial cells. J Exp Med 2000;191:593–602.

13 Higashi H, Tsutsumi R, Muto S, Sugiyama T, Azuma T, Asaka M, Hatakeyama M: SHP-2 tyrosine phosphatase as an intracellular target of *Helicobacter pylori* cagA protein. Science 2002;295:683–686.

14 Higashi H, Tsutsumi R, Fujita A, Yamazaki S, Asaka M, Azuma T, Hatakeyama M: Biological activity of the *Helicobacter pylori* virulence factor cagA is determined by variation in the tyrosine phosphorylation sites. Proc Natl Acad Sci USA 2002;99:14428–14433.

15 Azuma T, Yamazaki S, Yamakawa A, Ohtani M, Muramatsu A, Suto H, Ito Y, Dojo M, Yamazaki Y, Kuriyama M, Keida Y, Higashi H, Hatakeyama M: Association between diversity in the Src homology 2 domain-containing tyrosine phosphatase binding site of *Helicobacter pylori* cagA protein and gastric atrophy and cancer. J Infect Dis 2004;189:820–827.

16 Dixon MF, Genta RM, Yardley JH, Correa P: Classification and grading of gastritis. The updated Sydney system. International workshop on the histopathology of gastritis, Houston 1994. Am J Surg Pathol 1996;20:1161–1181.

17 Hatakeyama M: Oncogenic mechanisms of the *Helicobacter pylori* cagA protein. Nat Rev Cancer 2004;4:688–694.

18 Maeda S, Yoshida H, Ikenoue T, Ogura K, Kanai F, Kato N, Shiratori Y, Omata M: Structure of *cag* pathogenicity island in Japanese *Helicobacter pylori* isolates. Gut 1999;44:336–341.

19 Azuma T, Yamakawa A, Yamazaki S, Ohtani M, Ito Y, Muramatsu A, Suto H, Yamazaki Y, Keida Y, Higashi H, Hatakeyama M: Distinct diversity of the *cag* pathogenicity island among *Helicobacter pylori* strains in Japan. J Clin Microbiol 2004;42:2508–2517.

20 Backstrom A, Lundberg C, Kersulyte D, Berg DE, Boren T, Arnqvist A: Metastability of *Helicobacter pylori* bab adhesin genes and dynamics in Lewis b antigen binding. Proc Natl Acad Sci USA 2004;101:16923–16928.

21 Haruma K, Komoto K, Kamada T, Ito M, Kitadai Y, Yoshihara M, Sumii K, Kajiyama G: *Helicobacter pylori* is a major risk factor for gastric carcinoma in young patients. Scand J Gastroenterol 2000;35:255–259.

22 Ito M, Haruma K, Kamada T, Kitadai Y, Hidaka T, Tsuda T, Komatsu H, Fukuhara T, Yoshihara M, Chayama K: Reduction in the incidence of *Helicobacter pylori*-associated carcinoma in Japanese young adults. Oncol Rep 2001;8:633–636.

23 Merrell DS, Goodrich ML, Otto G, Tompkins LS, Falkow S: pH-regulated gene expression of the gastric pathogen *Helicobacter pylori*. Infect Immun 2003;71:3529–3539.

24 Masuda H, Hiyama T, Yoshihara M, Tanaka S, Shimamoto F, Haruma K, Chayama K: Necessity of multiple gastric biopsies from different sites for detection of clarithromycin-resistant *Helicobacter pylori* strains. Scand J Gastroenterol 2003;38:942–946.

25 Scholte GH, van Doorn LJ, Cats A, Bloemena E, Lindeman J, Quint WG, Meuwissen SG, Kuipers EJ: Genotyping of *Helicobacter pylori* in paraffin-embedded gastric biopsy specimens: relation to histological parameters and effects on therapy. Am J Gastroenterol 2002;97:1687–1695.

26 Koehler CI, Mues MB, Dienes HP, Kriegsmann J, Schirmacher P, Odenthal M: *Helicobacter pylori* genotyping in gastric adenocarcinoma and MALT lymphoma by multiplex PCR analyses of paraffin wax embedded tissues. Mol Pathol 2003;56:36–42.

経 験

上部消化管内視鏡検診の現状および受診者側の期待度
―内視鏡検診の標準的方法の策定に向けて―

日山 亨，吉原 正治[1]，田中 信治[2]，伊藤 公訓，茶山 一彰[3]

1）広島大学保健管理センター
2）広島大学病院光学医療診療部
3）広島大学大学院分子病態制御内科学

〔要 旨〕

上部消化管内視鏡検診の標準的方法の作成に向けて，上部消化管内視鏡検診の実態ならびに受診者側の内視鏡検診に対する期待度について，アンケート調査を行った。対象は，任意に選んだ広島県内の医師71人およびコメディカルスタッフ55人，非医療従事者50人である。内視鏡検診の目的を，「内視鏡治療により治癒できる早期胃癌の発見」とした者が，医師，非医療従事者，コメディカルスタッフいずれも3人に2人以上と多数を占めた。また，受診者側が内視鏡検診に一番に望むことは，「検査を楽に」ということであった。しかし，非医療従事者とともにコメディカルスタッフも，内視鏡の診断能や偶発症などについて十分に理解しているとは言えず，内視鏡検診に関して更なる教育啓蒙活動が必要と考えられた。今後，内視鏡検診の実態と受診者側の期待を踏まえ，コンセンサスが得られる内視鏡検診の標準的方法を確立していく必要がある。

キーワード 内視鏡検診，アンケート，上部消化管

I はじめに

現在，人間ドックや個別検診などでは，内視鏡検査を用いた上部消化管検診の行われる機会が増えてきている[1]。しかし現時点では，その方法や内容には一定の基準はなく，精度管理上，上部消化管内視鏡検診の標準的方法を作成することが必要と思われる。そのために必要な検討項目には，(1) 対象年齢，(2) 間隔，(3) インフォームド・コンセントの内容，取得の方法，(4) 使用する内視鏡機器，(5) 前処置の方法，(6) 観察・撮影・記録方法，(7) 生検を実施するか否か，(8) 内視鏡の洗浄方法等の感染対策，(9) 画像のダブルチェックの実施が必要か否か，(10) 精度管理が必要か否か，などが挙げられよう。ただし，上部消化管内視鏡検診の標準的方法を策定するにあたっては，胃癌発生率等のエビデンスのみならず，現在の医療機関における上部消化管内視鏡検診の実態と受診者側の期待度についても考慮する必要がある。なぜなら，標準的方法は，多くの施設で実施可能なものでなければならず，また，検診の実態が受診者側の期待と大きく食い違うものであれば，当然，インフォームド・コンセント上問題であるし，受診率も上がらないという結果につながるからである。そこで，今回われわれは広島県における上部消化管内視鏡検診の実態および非医療従事者（受診者側）および看護師等のコメディカルスタッフにおける内視鏡検診に対する期待度について，アンケート調査を行った。その結果を踏まえ，内視鏡検診の標準的方法策定のための提言を行ったので，ここに報告する。

表1：アンケート調査の対象

・医師：71人

	20歳代	30歳代	40歳代	50歳代	60歳以上
人数	4	39	23	4	1

	診療所	個人病院	公的病院	健診センター
人数	13	31	20	7

・コメディカル：55人

	20歳代	30歳代	40歳代	50歳代	60歳以上
人数	9	25	12	8	1

	男	女
人数	8	47

	受診歴あり	受診歴なし
人数	35	20

・非医療従事者：50人

	20歳代	30歳代	40歳代	50歳代	60歳以上
人数	5	12	16	11	6

	男	女
人数	18	32

	受診歴あり	受診歴なし
人数	40	10

Ⅱ　対象と方法

今回，アンケート調査を行った対象は，任意に選んだ広島県内の上部消化管内視鏡検診業務に従事する医師71人（1施設1人）および看護師等のコメディカルスタッフ55人，非医療従事者（受診者側）50人である。その年齢，性別等の詳細を（表1）に示す。

次に，上部消化管内視鏡検診の実態を調べるために用いた医師向けのアンケートを（図1）に示す。質問1で，内視鏡検診の形態を尋ね，質問2で，内視鏡検診の主な目的が，「胃癌による死亡率の減少」，あるいは，「内視鏡治療により治癒できる早期胃癌の発見」，のいずれにあるかを尋ねた。質問3以降で，「はじめに」で挙げた各検討項目について尋ねた。

コメディカルスタッフおよび非医療従事者向けの，内視鏡検診に対する期待度を調べるために用いたアンケートを（図2）に示す。質問1，2は胃検診を受けたことのある人への質問事項，質問3～5は過去受診歴のない人への質問項目である。質問6～13までは内視鏡検診の理解度を問う質問である。胃X線検査との違いを尋ねることにより，内視鏡検診の理解度を問うた。質問14は，医師に行ったのと同様の内視鏡検診の目的に関することである。質問15では，今後の内視鏡検診に一番に望むことを尋ねた。

Ⅲ　結果

1. 医師に対するアンケートの結果　－上部消化管内視鏡検診の実態－

上部消化管内視鏡検診の目的について，「内視鏡治療により治癒できる早期胃癌の発見」と回答した医師が47人（66%）と多数を占めた。「胃癌による死亡率の低下」と回答した医師は22人（31%），回答なしは2人（3%）であった（図3左）。

検診の対象年齢は，40歳からという回答が約半数を占めた（図4）。「何歳まで？」という質問に対しては，80歳もしくはそれ以上と回答した医師が過半数を超えた（図5左）。

検診の間隔に関しては，1年に1回と回答した医師が65人（92%），2年に1回と回答した6

図1：アンケート用紙(医師向け)

質問1．貴病院では，上部消化管内視鏡検診を行っていますか？

①はい（形態は，①個別検診，②人間ドック，③施設検診），② いいえ

質問2．内視鏡検診の主な目的は，次のどれであるとお考えでしょうか？

① 胃癌による死亡率の減少，②内視鏡治療により治癒できる早期胃癌の発見，③その他（　　）

質問3．内視鏡検診を，何歳から受けるようすすめていますか？　（　　）歳から

質問4．内視鏡検診を，何歳まで受けるようすすめていますか？　（　　）歳くらいまで

質問5．内視鏡検診を，どれくらいの間隔で受けるようすすめていますか？

①1年に2回，② 1年に1回，③ 2年に1回，④ 3年に1回，⑤それ以外（ 年に1回）

質問6．ヘリコバクター・ピロリ未感染者は，ピロリ感染者に比べ胃癌発生率が有意に低いことが明らかになっています。ピロリ菌未感染の受診者の検診間隔を，延ばすことは可能だと思いますか？

①可能→検診間隔を（ ）年に1回に延ばすことができる

②検診間隔を延ばすべきではない　→　理由（　　）

質問7．内視鏡検診に先立って，検査のリスクも含めて説明し，文書への署名による同意を得ていますか？　　①はい，②いいえ

質問8．内視鏡検診に使用する内視鏡は次のどれですか？ ①普通径，② 細径，③経鼻・細径

質問9．内視鏡検診の前処置は通常次のどれで行っていますか？

① 咽頭麻酔，②鎮痙剤（ブスコパン，セスデン，グルカゴンなど），③鎮静剤（セルシン，ホリゾン，ドルミカム，オピスタンなど），④その他（　　）

質問10．内視鏡検診での写真撮影は通常何枚ですか？

① 10枚未満，②10枚以上20枚未満，③20枚以上30枚未満，④30枚以上

質問11．内視鏡画像は何に保存されていますか？

① フィルム，②MOなどの電子メディア，③ファイリングシステム，④その他（　）

質問12．内視鏡検診で病変（疑）を発見した時，生検は行っていますか？ ①はい，②いいえ

質問13．生検鉗子は，①ディスポーザブル製品，②繰り返し滅菌したもの

質問14．内視鏡の洗浄は，①毎回器械洗浄，②毎回手洗いで，最後に器械洗浄，③手洗いのみ

質問15．内視鏡画像のダブルチェックは行っていますか？ ①はい，②いいえ

質問16．精度管理（見逃し例のチェックなど）は行っていますか？ ①はい，②いいえ

図2：アンケート用紙(非医療従事者およびコメディカルスタッフ向け)

◇胃の検診を受けたことがある方

質問1. 胃の検診は,何歳から受けはじめましたか? ( )歳から

質問2. 胃の検診は何歳くらいまで受けようと思いますか? ( )歳くらいまで

質問11へ行ってください。

◇これまで胃の検診を受けたことがない方

質問3. どうして胃の検診を受けなかったのですか? 一番あてはまるものを一つ選んでください。

①検診の対象年齢ではなかったので,②胃の調子は,特に悪くないから,③検査が苦しいから,④費用が高いから,⑤検査により偶発症(不利益)を生じることあるから,⑥その他( )

質問4. 胃の検診はいつから受けようと思いますか?

①( )歳ころから,②胃の調子が悪いなどの症状があったら

質問5. 胃の検診は何歳くらいまで受けようと思いますか? ( )歳くらいまで

◇全員にお願いします

内視鏡(胃カメラ)検査はX線(バリウム)検査に比べ,

	まったくそう思わない	あまりそう思わない	どちらでもない	少しそう思う	とてもそう思う
質問6. 検査が楽である					
質問7. 検査時間が短い					
質問8. 費用が安い					
質問9. 偶発症(不利益)が少ない					
質問10. 感染の危険が少ない					
質問11. 進行した胃がんの見逃しが少ない					
質問12. 早期の胃がんも発見できる					
質問13. 胃がん以外の疾患の診断も正確にできる					

質問14. 胃の内視鏡検診の主な目的は次のどれであるとお考えでしょうか?

①胃癌で死亡する人を減らす,②内視鏡治療で治る早期胃癌の発見, ③その他( )

質問15. 今後,胃の内視鏡検診に一番に望むことは何ですか?

①検査を楽にして欲しい,②検査時間を短くして欲しい,③費用を安くして欲しい,④地域・職場の検診に組み込んで欲しい,⑤検査の偶発症(不利益)を少なくして欲しい,⑥感染の危険をなくして欲しい,⑦胃がんの見逃しをなくして欲しい,⑧できるだけ早期の段階で胃がんを発見して欲しい,⑨胃がん以外の疾患の診断もより正確にして欲しい,⑩その他( )