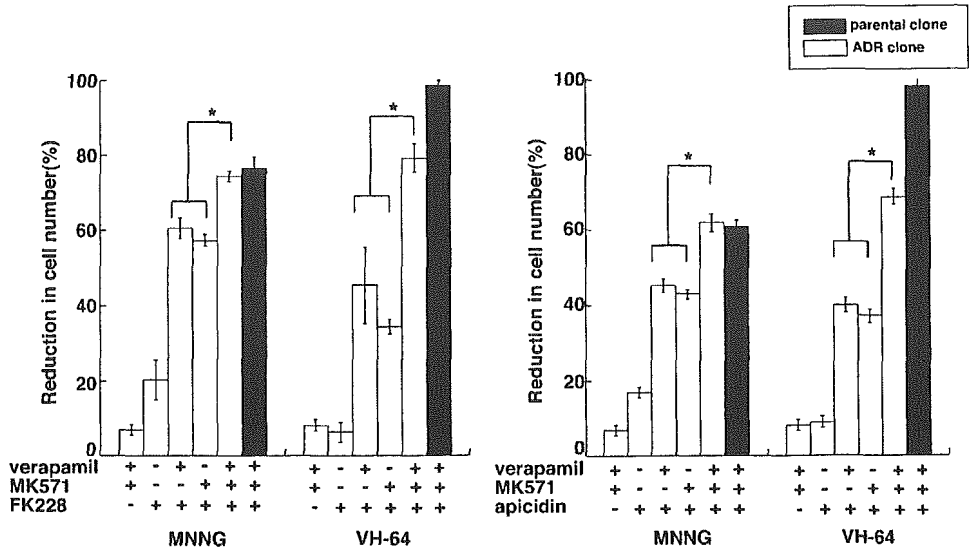

FIGURE 6 – Effects of the combination of verapamil and MK571 on the drug activities of FK228 (left panel) and apicidin (right panel) in the parental and ADR clones (black bars, parental clones; white bars, ADR clones). After the cells were incubated with 100 ng/ml FK228 and 3.2 μM apicidin for 48 hr in the presence of 10 μM verapamil and/or 50 μM MK571, cell death was determined by a CellTiter-Glo™ Luminescent Cell Viability Assay. The data represent the means of three separate experiments performed in triplicate; bars represent SD. Statistical differences between samples ($p < 0.001$) were determined by repeated measure ANOVA with Scheffe post-hoc test and are denoted by an asterisk.

We also found that FK228 and apicidin might be the substrates of P-gp and MRP1. P-gp and MRP1 may contribute to the resistance mechanisms against FK228 and apicidin in ADR clones. Our data demonstrated that both P-gp inhibitor verapamil and MRP1 inhibitor MK571 enhanced cell death, apoptosis and accumulation of Ac-H3 by FK228 and apicidin in ADR clones. The results are consistent with a previous study reporting that FK228 was a substrate of P-gp.[31] However, to our knowledge, our study is the first to indicate the involvement of MRP1 in the mechanisms of resistance against FK228. Furthermore, there is no previous study analyzing the mechanisms of resistance against apicidin. A number of HDACIs have been reported and can be classified according to their chemical nature and mechanisms of inhibition. For instance, Valproic acid inhibits proliferation and induces apoptosis in acute myeloid leukemia cells expressing P-gp and MRP1.[32] Trichostatin A and Suberoyllanilide Hydroxamic acid (SAHA) markedly reduce cell viability and promote apoptosis in drug-resistant cells expressing P-gp.[29,33,34] HL60/ADR, the doxorubicin-resistant clone of HL60 obtained by the transfection of the *MDR-1* gene, was equally sensitive to pivaloyloxymethyl butylate-induced cytotoxicity as the parental cells.[35] Overall, these data suggest that several types of HDACIs can overcome the drug resistance of the cells expressing P-gp and/or MRP1. However, we revealed that FK228 and apicidin showed strong cross-resistance against the drug-resistant clones expressing P-gp and MRP1, which independently contributed to their resistance mechanisms. It is noteworthy that both FK228 and apicidin belong to the cyclic tetrapeptide family and have similar structural characteristics such as cyclic tetrapeptide scaffolds and similar molecular weights (FK228: 540.71, apicidin: 623.8). It is well known that typical multidrug resistance is caused by both Pgp and MRP1 and that MRP1 has similar transport specificity as P-gp.[36] Therefore, in ADR clones of OS and EFTs, FK228 and apicidin might be common substrates of Pgp and MRP1 probably because of the structural similarities of the drugs as cyclic tetrapeptides.

As mentioned above, FK228 and apicidn have several similarities; however, there was a clear difference in the levels of resistance between FK228 and apicidin (Table I, Fig. 2*c* and Fig. 3). Apicidin exhibited the cytotoxic effects at "μM" order, whereas FK228 exhibited at "pM (ng/ml)" order in parental clones (Fig. 1). Thus, apicidin exhibited cytotoxicity at higher concentrations than FK228 did. P-gp and MRP1 work as efflux pumps in ADR clones, and the efflux pumps have the limitation of ability to pump off the agents. If efflux pumps could pump off the agents at "mM" order in ADR clones, for instance, the difference between parental clones and ADR clones in IC50 of apicidin would be more than that of FK228, since the fold resistance of apicidin might be represented as the ratio of "mM" against "μM", whereas the fold resistance of FK228 might be represented as the ratio of "mM" against "pM". In fact, fold resistance of apicidin was from about 3 to about 30, whereas that of FK228 was from about 300 to about 600 (Table I).

FIGURE 4 – Effects of Pgp inhibitor verapamil on drug activities of FK228 and apicidin in the parental and ADR clones. (*a*) The expression of Pgp in the parental and ADR clones was examined by Western blot analysis using an antibody against P-glycoprotein (170 kD). The actin blot was performed as loading control. (*b*) Cells were incubated with 100 ng/ml FK228 and 3.2 μM apicidin for 48 hr in the presence or absence of 10 μM verapamil (black bars, parental clones; white bars, ADR clones), then the percentage of cell death was determined by the CellTiter-Glo™ Luminescent Cell Viability Assay. The data represent the means of three separate experiments performed in triplicate; bars represent SD. Statistical differences between samples ($p < 0.001$) were determined by repeated measure ANOVA with Scheffe post-hoc test and are denoted by an asterisk. (*c*) Cells were incubated with various concentrations of FK228 and apicidin for 24 hr in the presence or absence of 10 μM verapamil. Whole cell fractions were isolated, and accumulation of acetylated histone H3 was examined by Western blot analysis using antibodies against acetylated histone H3 (17 kD). The actin blot was performed as loading control. (*d*) Cells were incubated with various concentrations of FK228 and apicidin for 24 hr in the presence or absence of 10 μM verapamil. The expression of cleaved-PARP was detected using antibodies against the cleavage form of PARP (85 kD). The actin blot was performed as loading control.

FIGURE 5 – Effect of MRP1 inhibitor MK571 on the drug activities of FK228 and apicidin in the parental clones and in ADR clones. (*a*) The expression of MRP1 in the parental and ADR clones was examined by Western blot analysis using antibodies against MRP1 (190 kD). The actin blot was performed as loading control. (*b*) Cells were incubated with 100 ng/ml FK228 and 3.2 μM apicidin for 48 hr in the presence or absence of 50 μM MK571 (black bars, parental clones; white bars, ADR clones), then the percentage of cell death was determined by a CellTiter-Glo™ Luminescent Cell Viability Assay. The data represent the means of three separate experiments performed in triplicate; bars represent SD. Statistical differences between samples ($p < 0.001$) were determined by repeated measure ANOVA with Scheffe post-hoc test and are denoted by an asterisk. (*c*) Cells were incubated with various concentrations of FK228 and apicidin for 24 hr in the presence or absence of 50 μM MK571. Whole cell fractions were isolated, and the accumulation of acetylated histone H3 was examined by Western blot analysis using anti-acetylated histone H3 antibodies (17 kD). The actin blot was performed as loading control. (*d*) Cells were incubated with various concentrations of FK228 and apicidin for 24 hr in the presence or absence of 50 μM MK571. The cleaved form of PARP was detected using anti-cleaved PARP antibodies (85 kD). The actin blot was performed as loading control.

With respect to the dominance effects of P-gp and MRP1, P-gp and MRP1 seem to independently contribute to the resistance against FK228 and apicidin. Roland *et al.* reported that although MRP might contribute to gemtuzumab ozogamicin (a humanized anti-CD33 antibody conjugated to a calicheamicin derivative) resistance, the inhibition of P-gp function was required to reveal an effect of MRP on the resistance to gemtuzumab ozogamicin in acute myeloid leukemia cell lines expressing P-gp and MRP1.[37] In our present study, however, we show that verapamil alone and MK571 alone could independently reverse the resistance of FK228 and apicidin. Moreover, we found that the combination of verapamil and MK571 further enhanced FK228 and apicidin-induced cell death more than verapamil alone or MK571 alone. These observations implicate that MRP1 as well as P-gp independently contribute to the resistance mechanisms in OS and EFTs ADR clones expressing P-gp and MRP1, suggesting the importance of the combination of these inhibitors of efflux pumps in the use for the drug-resistant tumors expressing P-gp and MRP1.

In summary, we demonstrated that P-gp and MRP1 contributed to the resistance mechanisms against cyclic tetrapeptide HDACIs FK228 and apicidin since both HDACIs were substrates of the efflux pumps. Although it will be possible to treat these tumors with other HDACIs that are not substrates of P-gp and/or MRP1, these findings suggest that it may be difficult to treat tumors expressing P-gp and/or MRP1 with these HDACIs being substrates of them. Our present study provides new insights into the indication for the use of the cyclic tetrapeptide HDACIs FK228 and apicidin to treat malignant tumors. Since HDACIs are potent and promising antitumor drugs and seem to be close to clinical use, it is important to clarify the expression of P-gp and MRP1 in patients' tumors.

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Long-term Clinical Outcome of *Helicobacter pylori* Eradication for Gastric Mucosa-Associated Lymphoid Tissue Lymphoma with a Reference to Second-Line Treatment

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BACKGROUND. The goals of the current study were to elucidate the long-term outcome of *Helicobacter pylori* eradication therapy for gastric mucosa-associated lymphoid tissue (MALT) lymphoma and to clarify the therapeutic efficacy of stomach-conserving treatments for patients not responding to eradication therapy.

METHODS. Ninety-six patients with gastric MALT lymphoma, including 17 patients with areas of diffuse large B-cell lymphoma, were treated by *H. pylori* eradication. Patients not responding to eradication therapy underwent either a gastrectomy, multiagent chemotherapy, oral monochemotherapy (OMC), or radiotherapy (RT). Predictive factors for the response to eradication therapy, overall survival (OS), and event-free survival (EFS) were determined by the Kaplan–Meier analysis with the log-rank test. The efficacy of second-line treatment was compared between OMC and RT.

RESULTS. After eradication therapy, 62 (65%) patients achieved complete disease remission (CR). Transient histologic disease recurrence was confirmed in 4 (6.5%) of 62 patients with CR during the follow-up (median, 37.5 months). The OS and EFS probabilities after 5 years were 0.96 and 0.80, respectively. Second-line treatment was performed in 31 patients; gastrectomy in 4 patients, multiagent chemotherapy in 5 patients, OMC in 12 patients, and RT in 10 patients. There were no differences in the CR rate, OS, EFS, or toxicity between the OMC and RT groups.

CONCLUSIONS. *H. pylori* eradication therapy was an effective first-line treatment for patients with gastric MALT lymphoma, which led to a favorable long-term outcome. OMC and RT had an equivalent efficacy as a second-line treatment in nonresponding patients to eradication therapy. ***Cancer* 2005;104:532–40.**

KEYWORDS: gastric lymphoma, mucosa-associated lymphoid tissue, *Helicobacter pylori*, chemotherapy, radiotherapy.

Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) is a distinct clinicopathologic entity in the recent classifications of malignant lymphomas.[1,2] *Helicobacter pylori* has been shown clearly to play a causative role in the pathogenesis of gastric MALT lymphoma, and the eradication of *H. pylori* thus leads to a regression of lymphoma in a certain proportion of patients.[3–6] Approximately 60–90% of patients with gastric MALT lymphoma have been reported to achieve complete disease remission (CR) after *H. pylori* eradication therapy.[5–15] Patients not responding to eradication therapy have been characterized by *H. pylori* negative patients, presence of the diffuse large B-cell lymphoma (DLBL) com-

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ponent, advanced disease stage, deep tumor invasion into the gastric wall as determined by endoscopic ultrasonography (EUS), or chromosomal translocation t(11; 18) (q21; q21).[6–13] However, there were few patients in the previous series, and data on the long-term follow-up after eradication therapy are rare.[14,15]

Conversely, the therapeutic strategy for gastric MALT lymphoma that does not respond to *H. pylori* eradication therapy still remains to be elucidated. In the past, the majority of such patients tended to be treated by surgical resection.[16–18] In recent years, however, stomach-conserving treatments such as chemotherapy or irradiation have become increasingly popular even for patients with resectable tumors. Although the efficacy of single-agent chemotherapy/oral monochemotherapy (OMC)[19,20] and radiotherapy (RT)[21–23] for gastric MALT lymphoma has been described, no previous reports have yet compared these modalities in detail.

We previously reported the outcome of the initial response to *H. pylori* eradication in 41 patients with gastric MALT lymphoma, in whom EUS was predictive for the effectiveness of this therapy.[12] The aims of the current study were 1) to elucidate the long-term outcome of *H. pylori* eradication therapy in a large cohort of patients with gastric MALT lymphoma with a reassessment of the predictive factors for CR and 2) to clarify the therapeutic efficacy of stomach-conserving treatments for patients refractory to eradication therapy, with special reference to the comparison of OMC and RT.

MATERIALS AND METHODS

Subjects

From 1994 to 2003, 96 patients with gastric MALT lymphoma with or without areas of DLBL were enrolled in our prospective study on the therapeutic efficacy of *H. pylori* eradication. The patients included 45 men and 51 women with a mean age of 61.7 years (range, 16–84 years) at study entry. The results of a preliminary analysis of 41 of these patients were described previously.[12] The histologic diagnosis of lymphoma was based on an evaluation of the biopsy and/or endoscopically resected specimens by two observers (Shotaro N and TY), according to the World Health Organization classification.[2] All tissue specimens underwent immunohistochemical staining for CD3 and CD 20/CD79a. Low-grade MALT lymphoma was defined as a diffuse proliferation of centrocyte-like cells with lymphoepithelial lesions.[1,2] A diagnosis of DLBL was based on the presence of solid or sheet-like proliferations of large neoplastic B cells.[1,2] Seventy-nine patients were diagnosed with low-grade MALT lymphoma, whereas the remaining 17 patients had MALT lymphoma plus DLBL (formerly referred to as high-grade MALT lymphoma).

The staging workup included a physical examination with an inspection of Waldeyer's tonsillar ring, blood cell count and serum chemistry analysis, chest radiographs, abdominal ultrasound, computed tomography (CT) scans of the chest and abdomen, colonoscopy, small bowel barium radiography, bone marrow aspiration or biopsy, and gallium scintigraphy or fluorine-18 fluorodeoxyglucose positron emission tomography.[24] EUS was performed to evaluate the depth of tumor invasion and the degree of perigastric lymphadenopathy in all patients.[12] According to the Lugano International Conference classification for gastrointestinal lymphoma,[25] 79 patients were classified with Stage I disease, 11 with Stage II_1 disease, and 6 with Stage IVdisease. Eighty-nine patients were positive for *H. pylori* infection and 7 patients were negative for *H. pylori* infection, as determined by histology, culture, rapid urease test, ^{13}C urea breath test, and/or serology. The API2-MALT1 chimeric transcript, which is specific to the chromosomal translocation t(11; 18) (q21; q21), was investigated in 50 patients using fresh or frozen tissue samples by means of a reverse transcription polymerase chain reaction (PCR), as described previously.[26] In these 50 patients, B-cell monoclonality was confirmed by PCR for immunoglobulin heavy chain gene rearrangement.[27]

The current study was carried out in accordance with the Helsinki Declaration as revised in 1989. Informed consent was obtained from each patient with regard to the aims and protocol of the study.

Helicobacter pylori Eradication Therapy

As the first-line treatment, all 96 patients, including 7 patients without *H. pylori* infection, received *H. pylori* eradication therapy with a proton pump inhibitor (40 mg/day omeprazole, 60 mg/day lansoprazole, or 20 or 40 mg/day rabeprazole) and a combination of antibiotics (1500 mg/day amoxicillin plus 600 mg/day clarithromycin with or without 750 mg/day metronidazole) for 14 days. In 7 patients, an additional regimen of high-dose dual therapy with oral administration of amoxicillin (500 mg/day) plus either lansoprazole (30 mg/day) or rabeprazole (10 mg/day) for 14 days was necessary to cure the infection. Successful eradication of *H. pylori* infection was achieved in all 89 patients who were initially positive for *H. pylori* infection, as determined by both ^{13}C urea breath test and histology. Follow-up endoscopic examinations with biopsies were performed every 4–8 weeks until confirmation of CR, and they were repeated every 3–6 months after CR. CT scans of the abdomen and/or chest were performed every 6 months when extragastric involvement

was recognized at the initial staging. CR was defined as the complete disappearance of clinical evidence of lymphoma and an absence of lymphoma cells on endoscopic biopsy specimens. Partial disease remission (PR) was defined as a tumor reduction of ≥ 50%. Patients showing neither CR nor PR (no response [NR]) and those exhibiting disease progression (PD) were considered to have failed to respond to the eradication therapy.

Second-Line Treatment

Second-line treatment was indicated in patients who failed to respond to eradication therapy. The decision of failure of response to *H. pylori* eradication therapy was made at 2 months for patients with MALT lymphoma plus DLBL and for those with PD, at 6 months for those without DLBL who showed NR, and at 12 months for those without DLBL who showed PR. Until 1997, patients who did not respond to eradication therapy underwent total gastrectomy. After 1998, patients not responding to eradication therapy were subjected to nonsurgical treatments, including chemotherapy and RT. Multiagent chemotherapy with the cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP)-based regimen with or without a monoclonal anti-CD20 antibody (rituximab)[28,29] was administered to patients with PD or with Stage IV disease. Other patients received either OMC or RT, which was dependent on the patients' choice. For OMC, 100 mg/day cyclophosphamide was administered for 6–12 months.[19,20] For RT, 30 gray (Gy) in 20 fractions (during the course of 4 weeks) was administered to the involved field for low-grade MALT lymphoma, and 40 Gy in 25–27 fractions (5–6 weeks) was administered for MALT lymphoma plus DLBL.[21–23] Toxicity of the second-line treatment was graded according to the National Cancer Institute' Common Toxicity Criteria, version 2.0.

Statistical Analysis

The probabilities of CR after *H. pylori* eradication therapy, and overall survival (OS) and event-free survival (EFS) were calculated by the Kaplan–Meier method, and the value was compared using the log-rank test. OS was measured from the date of the start of the treatment to death from any cause, and EFS was measured from the date of the start of the treatment to disease progression, disease recurrence, or death from any cause. All variables that influenced the EFS by the log-rank test ($P < 0.01$) were put into a multivariate analysis using the Cox proportional hazards model. Other statistical differences were evaluated using either the Fisher exact probability test, the chi-square test, or the Mann–Whitney U test. A value of $P < 0.05$ for each test was regarded as statistically significant. All statistical analyses were performed using the Statistical Software Package for the Social Sciences, version 8.0J (SPSS Japan Inc., Tokyo, Japan).

TABLE 1
Predicting Factors for CR of MALT Lymphoma after *H. pylori* Eradication Therapy Determined by Kaplan–Meier Analysis

Characteristics	No. of patients with CR (%)	Probability of CR at 1 yr	*P* value[a]
Depth of invasion by EUS			
Mucosa (n = 55)	52 (95)	0.87	<0.0001
SM or beyond (n = 41)	10 (24)	0.54	
Dominant site of lesion			
Proximal third (n = 34)	17 (50)	0.68	0.056
Distal two thirds (n = 62)	45 (73)	0.84	
Endoscopic appearance			
Superficial type (n = 65)	54 (83)	0.82	0.067
Other types (n = 31)	8 (26)	0.48	
H. pylori infection			
Positive (n = 89)	61 (69)	0.80	0.16
Negative (n = 7)	1 (14)	0.14	
Clinical stage			
I (n = 79)	56 (71)	0.81	0.24
II_1/IV (n = 17)	6 (35)	0.49	
DLBL component			
Yes (n = 17)	6 (35)	NA[c]	0.98
No (n = 79)	56 (71)	0.77	
API2-MALT1 transcript[b]			
Positive (n = 3)	0	0	0.21
Negative (n = 47)	29 (60)	0.79	

H. pylori: Helicobacter pylori; EUS: endoscopic ultrasonography; CR: complete disease remission; MALT: mucosa-associated lymphoid tissue; SM: submucosa; DLBL: diffuse large B-cell lymphoma, NA: not available.

[a] Log-rank test.

[b] n = 50.

[c] All patients without CR underwent second-line therapy within 6 months.

RESULTS

Outcome of Eradication Therapy

The median follow-up period after *H. pylori* eradication therapy was 38 months (range, 3–119 months). After eradication therapy, 62 (65%) patients achieved CR and 4 (4%) patients achieved PR, whereas 30 patients (31%) had NR or PD. The CR rate for patients with low-grade MALT lymphoma was 71% (56 of 79 patients), whereas the CR rate for patients with MALT lymphoma plus DLBL was 35% (6 of 17 patients). The median time interval between eradication therapy and a confirmation of CR was 2.5 months (range, 1–20 months).

Table 1 summarizes the probabilities of CR stratified by clinicopathologic factors. The depth of tumor invasion determined by EUS strongly correlated with the outcome of eradication therapy. CR was observed

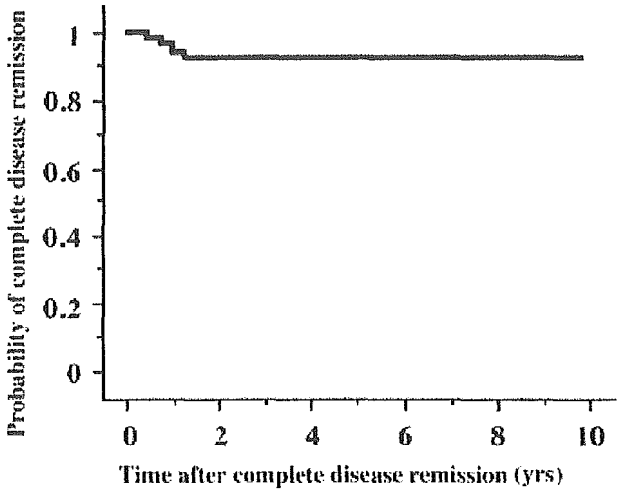

FIGURE 1. The survival curve for duration of complete disease remission (CR) for 62 patients with gastric mucosa-associated lymphoid tissue lymphoma who achieved CR after *Helicobacter pylori* eradication therapy. Local disease recurrence was found in four patients. The probability of CR after 5 years is 0.93 using the Kaplan–Meier analysis.

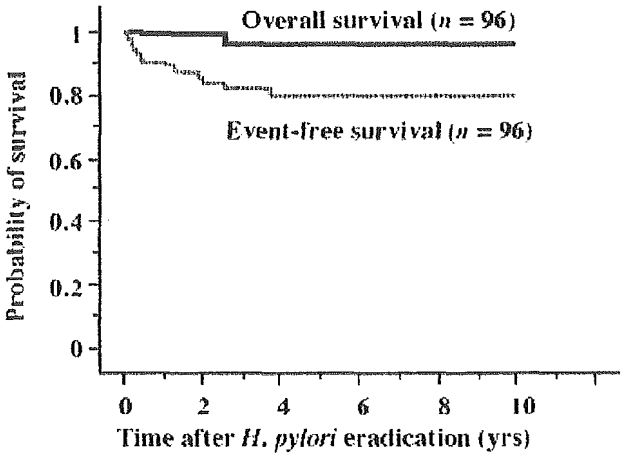

FIGURE 2. The overall survival (OS) and event-free survival (EFS) curves for all 96 patients with gastric mucosa-associated lymphoid tissue lymphoma after *Helicobacter pylori* eradication therapy. The OS and EFS probabilities after 5 years are 0.96 and 0.80, respectively.

in 52 of 55 (95%) patients with MALT lymphoma restricted to the mucosa, but only in 10 of 41 (24%) patients with tumors that had invaded the submucosa or beyond (log-rank test, $P < 0.0001$). Other factors, including the dominant site of involvement, endoscopic appearance, *H. pylori* infection status, clinical stage, DLBL component, or API2-MALT1 chimeric transcript, did not influence significantly the probability of CR.

The median follow-up period after CR in 62 patients was 37.5 months (range, 2–118 months). During the follow-up period, only 1 patient died of cirrhosis 30 months after CR. Secondary malignancy was not detected in these 62 patients. Local histologic disease recurrence of MALT lymphoma was found in 4 (6.5%) patients, 5, 9, 12, and 15 months, respectively, after CR (Fig. 1). At the time of disease recurrence, these four patients showed neither *H. pylori* reinfection, histology of DLBL, nor endoscopic sign of disease recurrence. The recurrent lymphoma cells in these patients spontaneously disappeared after 6 or 12 months without any additional treatment.

Of the 34 patients who did not achieve CR after *H. pylori* eradication therapy, 31 underwent second-line treatment. The other 3 patients, 1 with PR 2 months after eradication therapy and the other 2 with NR 5 and 6 months after eradication, have all been observed carefully.

Figure 2 demonstrates the survival curves for all 96 patients after eradication therapy. The OS and EFS probabilities after 5 years were 0.96 and 0.80, respectively. Table 2 summarizes the clinicopathologic factors that infuluenced the EFS. Based on the univariate analysis, the depth of invasion determined by EUS, the presence or absence of the DLBL component, and age of patients were significant variables that affected the EFS (Fig. 3). Endoscopic appearance of the superficial type showed better EFS than other types, but the difference was not statistically significant. Neither gender, dominant site, *H. pylori* infection status, clinical

TABLE 2
Prognostic Factors for EFS in 96 Patients with Gastric MALT Lymphoma after *H. pylori* Eradication

	Univariate[a]		Multivariate[c]	
Characteristics	**5-yr EFS**	***P* value[b]**	**Coefficient/SE**	***P* value**
Depth by EUS				
Mucosa (n = 55)	0.89	0.010	2.40	0.016
SM or beyond (n = 41)	0.68			
DLBL component				
Yes (n = 17)	0.63	0.0076	1.41	0.16
No (n = 79)	0.83			
Age (yrs)				
≤ 61 (n = 42)	0.90	0.016	0.44	0.66
≥ 62 (n = 54)	0.71			
Endoscopic type				
Superficial type (n = 65)	0.86	0.061	0.44	0.67
Other types (n = 31)	0.68			

SE: standard error; SM: submucosa; DLBL: diffuse large B-cell lymphoma; EFS: event-free survival; *H. pylori*: *Helicobacter pylori*; EUS: endoscopic ultrasonography; MALT: mucosa-associated lymphoid tissue; yrs: years.

[a] Kaplan–Meier analysis.

[b] Log-rank test.

[c] Cox proportional hazards model.

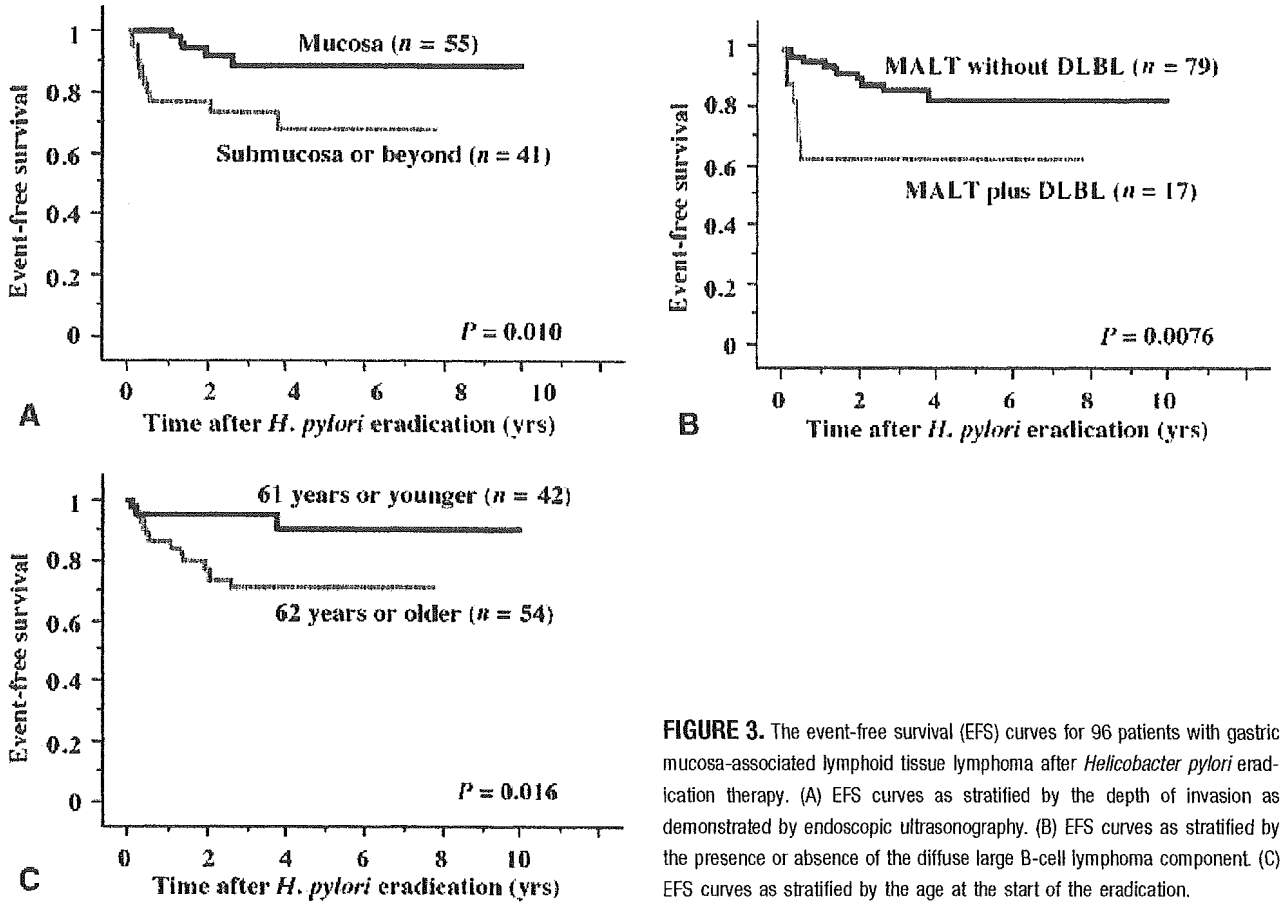

FIGURE 3. The event-free survival (EFS) curves for 96 patients with gastric mucosa-associated lymphoid tissue lymphoma after *Helicobacter pylori* eradication therapy. (A) EFS curves as stratified by the depth of invasion as demonstrated by endoscopic ultrasonography. (B) EFS curves as stratified by the presence or absence of the diffuse large B-cell lymphoma component. (C) EFS curves as stratified by the age at the start of the eradication.

stage, nor API2-MALT1 chimeric transcript affected the EFS. Multivariate analysis revealed the depth of invasion by EUS to be the only significant prognostic factor for the EFS (Table 2).

Outcome of Second-Line Treatment

As second-line therapy, a surgical resection (total gastrectomy) was performed in four patients. The other 27 patients underwent nonsurgical treatment. Five patients were treated with two to six cycles of CHOP based-chemotherapy. Three of the five patients were treated with a combination of CHOP and rituximab.[28,29] Twelve patients underwent OMC, whereas the other 10 received RT. Of the 31 patients who received second-line therapy, 27 patients (87%) achieved CR, but 4 patients (13%) showed NR or PD.

Between the patients treated by OMC (n = 12) and those treated by RT (n = 10), there were no significant differences in age, gender, the dominant site of the lesion (distal two-thirds in 5 of 12 [42%] who received OMC vs. 7 of 10 [70%] who received RT), endoscopic appearance (superficial type in 33% vs. 40%), presence of the DLBL component (25% vs. 50%), clinical stage (Stage I in 67% vs. 90%), or the depth of tumor invasion determined by EUS (submucosa or beyond in 92% vs. 90%). The median follow-up period after the therapy was not significantly different between the 2 groups (39.5 months in the OMC group and 32 months in the RT group). Treatment-related toxicity was generally mild, and no patients withdrew from the second-line therapy. Grade 3 or 4 toxicity was observed in 4 of 12 (33%) patients in the OMC group, and in 3 of 10 (30%) patients in the RT group (P = 0.62). In the OMC group, leukocytopenia was observed in 9 patients (1 in Grade 1, 4 in Grade 2, and 4 in Grade 3), Grade 1 or 2 nausea/emesis in 3 patients, and Grade 3 hemorrhagic cystitis in 1 patient. In the RT group, leukocytopenia was observed in 5 patients (2 in Grade 1, 1 in Grade 2, 1 in Grade 3, and 1 in Grade 4), and nausea/emesis was observed in 4 patients (2 in Grade 2, 2 in Grade 3).

Table 3 compares the efficacy of OMC and RT. CR was achieved in 10 of 12 (83%) patients in the OMC group (Fig. 4), and in 8 of 10 (80%) patients in the RT group (P = 0.63). In patients without DLBL, CR rates of OMC and RT were 89% (8 of 9 patients) and 100% (all 5 patients), respectively. Of the 18 patients with CR, an

TABLE 3
Comparison of the Efficacy of OMC and RT as Second-Line Treatment

Characteristics	OMC group (n = 12) (%)	RT group (n = 10) (%)	P value
Response to treatment			
CR	10 (83)	8 (80)	0.63[e]
NR/PD	2[a] (17)	2[b] (20)	
Disease recurrence after CR	1[b]	0	
Disease-free period (mos)	(n = 10)	(n = 8)	
Median	27.0	30.0	0.96[f]
Range	4–70	4–47	
Death	1[c] (8)	1[d] (10)	0.71[e]
3-yr survival probability			
Overall survival	0.88	0.90	0.82[g]
Event-free survival	0.79	0.80	0.81[g]

OMC: oral monochemotherapy; RT: radiotherapy; CR: complete disease remission; NR: no response; PD: progressive disease.
[a] One with diffuse large B-cell lymphoma (DLBL), one without DLBL.
[b] Positive for DLBL component.
[c] Pancreatic carcinoma.
[d] Heart failure.
[e] Fisher exact probability test.
[f] Mann-Whitney U test.
[g] Log-rank test.

OMC-treated patient with Stage II_1 MALT lymphoma plus DLBL had disease recurrence with bilateral hilar lymphadenopathy 24 months after CR. This patient was treated with four cycles of CHOP, which resulted in CR again. The disease-free period after the second-line treatment did not differ between the OMC and RT groups. A second malignancy was found in four patients during the follow-up. Of the patients in the OMC group, 1 patient was found to have pancreatic carcinoma 24 months after the start of OMC, whereas another patient had early gastric well differentiated adenocarcinoma after 42 months. The latter was treated successfully by an endoscopic mucosal resection. Among the RT group patients, 1 patient was diagnosed with myelodysplastic syndrome and another had early gastric poorly differentiated adenomacrinoma 6 months and 14 months after RT, respectively. The latter patient subsequently underwent a laparoscopy-assisted distal gastrectomy. Two patients died of causes unrelated to lymphoma. One died of pancreatic carcinoma 29 months after OMC, and the other of heart failure 3 months after RT. The probabilities of OS and EFS did not differ between the OMC and RT groups (Fig. 5).

Outcome of third-line treatment

Third-line treatment was administered to four patients who did not respond to the second-line treatment. All four patients were initially positive for *H. pylori* infection, which was eradicated successfully without any reinfection. One patient in the OMC group with MALT lymphoma plus DLBL in Stage I who showed PD at 3 months underwent a surgical resection, whereas the other patient in this group with low-grade MALT lymphoma in Stage I showing NR at

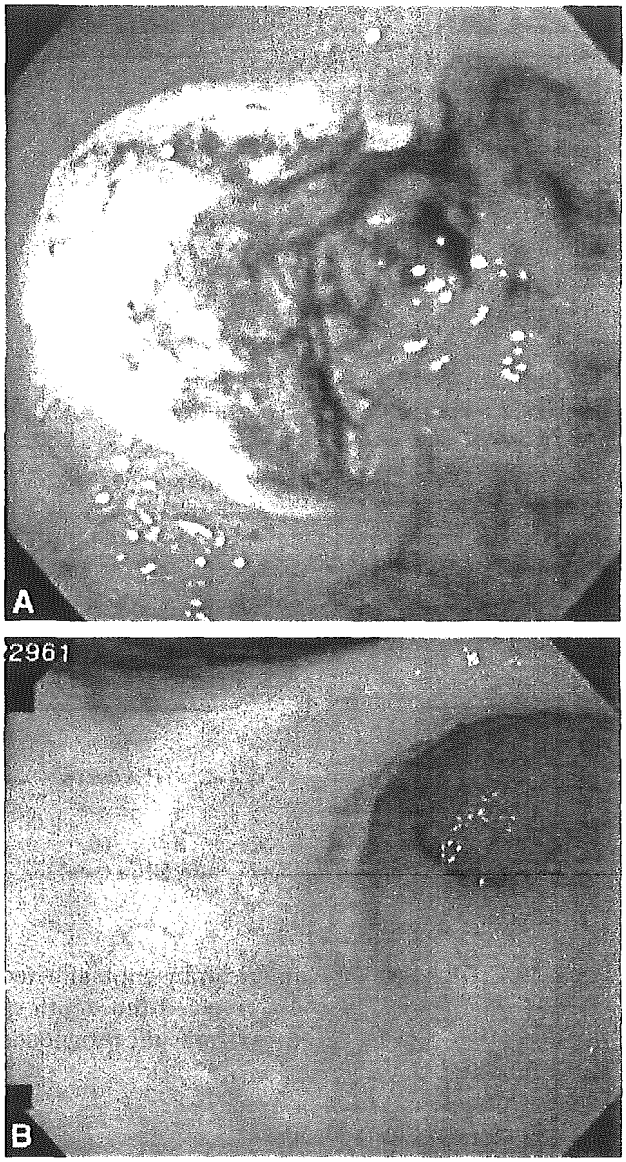

FIGURE 4. Patient with mucosa-associated lymphoid tissue lymphoma plus diffuse large B-cell lymphoma (Stage I) who achieved complete disease remission after oral monochemotherapy (OMC) with cyclophosphamide (72-year-old male patient). (A) An endoscopic picture 2 months after *Helicobacter pylori* eradication therapy. A large ulcerating tumor without a response to eradication therapy can be seen on the anterior wall of the antrum. (B) A follow-up endoscopic view 6 years after the completion of OMC reveals an almost normal mucosa without any evidence of lymphoma.

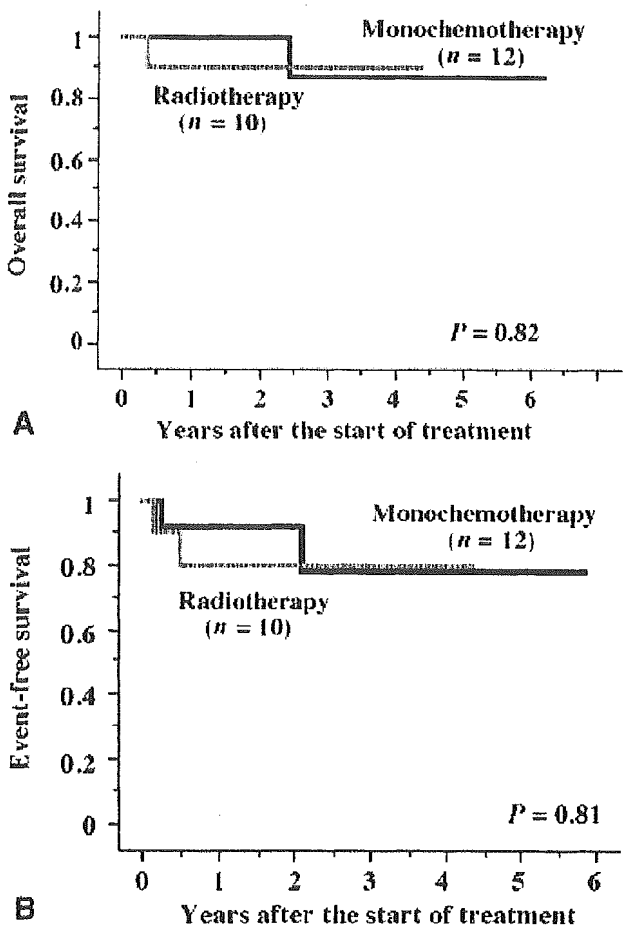

FIGURE 5. The survival curves for patients with gastric mucosa-associated lymphoid tissue lymphoma treated by second-line treatment with oral monochemotherapy (solid line; $n = 12$) and with radiotherapy (dotted line; $n = 10$). (A) Overall survival ($P = 0.82$). (B) Event-free survival ($P = 0.80$).

12 months underwent CHOP-based chemotherapy followed by RT with 30 Gy.[30] Two patients in the RT group with MALT lymphoma plus DLBL in Stage I showed PD with distant lymphadenopathy (one mesenteric, one hilar) at 2 months, and they subsequently underwent CHOP chemotherapy.

DISCUSSION

To date, a number of investigators have reported on the efficacy of *H. pylori* eradication therapy for patients with gastric MALT lymphoma.[5–15] Nowadays, eradication therapy has become the first choice of treatment for this disease. However, the number of long-term follow-up studies after *H. pylori* eradication therapy comprising a large cohort of patients with gastric MALT lymphoma is still insufficient. To our knowledge, there have been only 2 large series from Germany that have investigated > 80 patients ($n = 120$ and $n = 90$) for median follow-up periods of > 3 years.[14,15] The current study was the second largest series ($n = 96$) with a long-term follow-up period (median, 38 months). We demonstrated favorable prognosis as determined by OS and EFS in 96 patients with gastric MALT lymphoma after eradication therapy (Fig. 2).

In our previous study, the assessment of the invasion depth by EUS was the most critical factor for predicting the efficacy of eradication therapy in gastric MALT lymphoma.[12] In the current large study, we reconfirmed the predictive value of EUS for the response to *H. pylori* eradication therapy (Table 1). The EUS assessment for the depth of invasion also was found to be the only prognostic factor for EFS, as determined by multivariate analysis (Table 2). Some of the other factors, such as a dominant site, endoscopic appearance, *H. pylori* status, clinical stage, and DLBL component, were apparently associated with therapeutic efficacy, but the predictive values of such clinicopathologic characteristics were not statistically significant. Recent reports have shown that not a few patients with DLBL with or without areas of MALT lymphoma experienced disease remission after *H. pylori* eradication therapy.[12,31,32] In addition, 6 of 16 (38%) patients in Stage II_1/IV in our series achieved CR, even though such tumors in advanced stage have been reported to rarely respond to eradication therapy.[9,11,12]

The API2-MALT1 chimeric transcript mediated by t(11; 18) (q21; q21) translocation is one of the characteristic genetic alterations observed in a certain proportion of low-grade MALT lymphomas.[13,26,33] It has been clarified that most patients with gastric MALT lymphoma with this translocation do not respond to *H. pylori* eradication therapy.[13,26,33] In the current study, 3 patients positive for the API2-MALT1 chimeric transcript showed NR after eradication therapy. However, the chimeric transcript was not a significant predictive factor for a response to eradication therapy, probably due to its low prevalence in our series (6%) (Table 1).

There have been few publications describing disease recurrence after *H. pylori* eradication therapy in patients with gastric MALT lymphoma. The disease recurrence rate has been reported to vary from 7% to 12.5% in patients who once achieved CR.[8,10,14,15] The rate of disease recurrence in our study (4 of 62 patients [6.5%]) was similar to that reported by Savio et al.[10] (5 of 71 patients [7.0%]) and by Fischbach et al.[15] (4 of 56 patients [7.1%]). In the absence of the *H. pylori* reinfection or the DLBL component, such a histologic relapse is frequently a transient self-limiting event, as observed in our study and some previous reports.[10,34] It remains to be deter-

mined as to whether such a "self-limiting" disease recurrence is a true disease recurrence or an indication of minimal residual disease.[10,11,34]

There is currently no established consensus regarding the optimal second-line treatment for patients with gastric MALT lymphoma not responding to *H. pylori* eradication therapy. Several treatment modalities, including a surgical resection, chemotherapy, and RT, either alone or in combination, have been applied. In recent publications, stomach-conserving treatment, such as OMC or RT, has been a recommended strategy. In the current study, both OMC and RT showed excellent response rates without any differences in the OS, EFS, or furthermore, in the toxicity (Table 3). Thus, the efficacy of the two modalities seemed to be equivalent as a second-line therapy for gastric MALT lymphoma refractory to *H. pylori* eradication therapy, although our data had several limitations such as a small sample of patients, the heterogeneity with histology of lymphoma, and the lack of randomization.

In the literature, the CR rate after OMC was reported to range from 82% to 100% for patients with gastric low-grade MALT lymphoma in Stage I, and from 50% to 57% for those in Stage IV.[19,20] In the current study, we confirmed a similar CR rate (89%) in 9 patients including 3 patients with Stage II_1/IV low-grade lymphoma. In addition, 2 of 3 (67%) patients with DLBL also showed CR after OMC, although 1 of these 2 CR patients later experienced disease recurrence. Conversely, the CR rate of gastric MALT lymphoma after RT has been reported to range from 92% to 100% for patients without DLBL,[21–23] and 68% for those with DLBL.[21] The CR rate after RT in our study (100% for patients without DLBL, 60% for patients with DLBL) was also similar to the published data. However, it should be noted that 2 of the 5 (40%) patients with the DLBL component progressed to distant lymphadenopathy after RT. Taal et al.[21] reported that 9 of their 63 (14%) patients with high-grade gastric lymphoma (presumably equal to DLBL) treated by RT exhibited PD, and the lymphoma recurred in 19 of 50 (37%) patients who once achieved either CR or PR. Based on these observations, it can be considered that either OMC or RT is appropriate for localized (Stage I or II_1) low-grade MALT lymphoma without DLBL, whereas patients with the DLBL component should be treated by CHOP-based chemotherapy, rather than OMC or RT alone.

In our study, a second malignancy occurred in 2 of 12 (17%) patients after OMC and in 2 of 10 (20%) patients after RT. Although second cancers have been reported to be found in some patients with gastric MALT lymphoma, this incidence did not increase when compared with the general population.[35] Cyclophosphamide is a definitive carcinogenic agent for bladder carcinoma or leukemia, whereas its influence on the development of pancreatic and gastric carcinoma has not been verified. Although total body irradiation or a combination of RT and chemotherapy is well known to increase the risk of secondary myelodysplastic syndrome or acute leukemia, there is little evidence to suggest local RT alone is associated with an increased risk of these neoplasms.[36] Conversely, irradiation has been shown to increase the risk of secondary gastric carcinoma in patients with Hodgkin lymphoma[37] and in those with gastric lymphoma treated by a partial gastrectomy.[38] However, gastric carcinoma after RT in 1 of our patients may have been incidental, because the time interval between RT and the diagnosis of carcinoma in the patient (14 months) was extraordinarily short in comparison to previous data (4 to $\geq$ 10 years).[37,38]

Nevertheless, it still remains possible that in our patients OMC or RT may have contributed to the development of second malignancies to some degree. In our study, secondary gastric carcinoma was detected in 2 of 22 (9%) patients treated by OMC or RT, but in none of the 62 patients who achieved CR by *H. pylori* eradication therapy alone. In a large German study,[14] there were only 3 patients with gastric carcinoma among 97 patients with MALT lymphoma who achieved CR after *H. pylori* eradication therapy. In addition, the time interval from MALT lymphoma to gastric carcinoma exceeded > 4 years. These observations seem to suggest an association between second malignancies and OMC or RT, and this phenomenon needs to be further elucidated in the future.

In conclusion, *H. pylori* eradication therapy is an effective first-line treatment for gastric MALT lymphoma, which leads to a favorable long-term outcome. The depth of invasion under EUS is predictive of better EFS as well as a response to the eradication therapy. OMC and RT seem to have an equivalent efficacy as a second-line treatment for MALT lymphoma not responding to *H. pylori* eradication. However, longer follow-up studies in a large group of patients are still needed to clarify the long-term outcome and safety of these modalities.

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Original article

Relationship between biological behavior and phenotypic expression in undifferentiated-type gastric carcinomas

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Abstract

Background. **It has been proved that some differentiated-type gastric carcinomas have a gastric phenotype. Similarly, it can be conjectured that some undifferentiated-type gastric carcinomas have an intestinal phenotype and that there are biological differences between undifferentiated-type gastric carcinomas with a gastric phenotype and those with an intestinal phenotype. We classified the phenotypes of early undifferentiated-type gastric carcinomas and investigated the relationship between their biological behavior and the phenotypes.**

Methods. **Sixty lesions of intramucosal undifferentiated-type gastric carcinoma were classified into four phenotypes; gastric type, incomplete-intestinal type, complete-intestinal type, and unclassified type, according to the expression of CD10, MUC2, small-intestinal mucinous antigen (SIMA), human gastric mucin (HGM), or concanavalin A (ConA).**

Results. **The incidence of gastric-type carcinoma, incomplete-intestinal-type carcinoma, and complete-intestinal-type carcinoma was 33% (20 cases), 65% (39 cases), and 2% (1 case), respectively. There was no significant difference in any of the clinicopathological factors examined between the 20 gastric-type carcinomas and the 40 intestinal-type carcinomas, but there were significant differences in the morphological findings. Intestinal-type carcinomas had a glandular structure more frequently than the gastric-type carcinomas. The spreading pattern of gastric-type carcinomas showed a middle-layer type more frequently than the intestinal-type carcinomas.**

Conclusion. **Undifferentiated-type gastric carcinomas frequently expressed an intestinal phenotype. There were differences in the growth patterns between undifferentiated-type gastric carcinomas with a gastric phenotype and those with the intestinal phenotype.**

Key words Phenotypic expression · Undifferentiated-type gastric carcinomas · Growth pattern

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Introduction

Generally, gastric carcinomas are classified into two histological types by standard hematoxylin and eosin (H&E) staining; such as "intestinal" type and "diffuse" type, by Lauren [1], and "differentiated" type and "undifferentiated" type by Nakamura et al. [2]. It has been considered that intestinal-type carcinoma is similar to differentiated-type carcinoma and that diffuse-type carcinoma and undifferentiated-type carcinoma are similar and show a gastric phenotype. However, there are currently various opinions regarding the classification of gastric carcinoma phenotypes by mucin-histochemical or immunohistochemical methods. The existence of differentiated-type gastric carcinomas having a gastric phenotype has been proved. Tatematsu et al. [3] and Egashira [4] reported that about 30% of differentiated-type gastric carcinomas showed a gastric phenotype, by immunohistochemical or mucin-histochemical studies. We have reported that, in immunohistochemical studies, 38.8% of differentiated-type gastric carcinomas showed a gastric phenotype [5]. Generally, differentiated-type gastric carcinomas with a gastric phenotype are considered to have high invasiveness and high metastatic potential compared with differentiated-type gastric carcinomas with an intestinal phenotype [6–11]. It has been reported that there are undifferentiated-type gastric carcinomas having an intestinal phenotype [12,13]. Fiocca et al. [14] and Yamachika et al. [15] classified the phenotypes of gastric signet-ring cell carcinomas, and reported that the progression of gastric carcinomas was associated with a phenotypic shift from gastric type to intestinal type. Similarly, Yao et al. [16] reported that intestinalization frequently occurred during neoplastic transformation. It can be conjectured that there are biological differences between undifferentiated-type gastric carcinomas with a gastric phenotype and those with an intestinal phenotype. But there has been no report about comparisons of biological behav-

ior between undifferentiated-type gastric carcinomas having a gastric phenotype and those having an intestinal phenotype.

In the study, we report here, we classified the phenotypes of early undifferentiated-type gastric carcinomas according to the type of intestinal metaplasia [5] and investigated the relationship between their biological behavior and the phenotypes.

Materials and methods

Materials

From our pathological files of gastric specimens that had been surgically resected at Kyushu University Hospital and its affiliated hospitals, 60 lesions of intramucosal undifferentiated-type gastric carcinoma were randomly selected for the present study. Undifferentiated-type gastric carcinoma was defined as poorly differentiated adenocarcinoma or signet-ring cell carcinoma. All the lesions were cut into serial step sections of 3–4 mm in width, fixed in 10 % formalin solution, and embedded in paraffin. Macroscopic and histological evaluations were made according to the classification established by the Japanese Research Society for Gastric Cancer [17]. The macroscopic features were divided into three major types; type I (polypoid), type II (superficial), and type III (excavated). Type II was further divided into three subtypes: type IIa (elevated), type IIb (flat), and type IIc (depressed). Composite types were classified based on the predominant subtype; for example, type IIc+III was considered to be type IIc. The depth of invasion and histological grade were classified according to the predominant component.

Immunohistochemical staining

To classify the phenotypic expression, the expressions of CD10, MUC2, small intestinal mucinous antigen (SIMA), human gastric mucin (HGM), or concanavalin A (ConA) were investigated by immunohistochemical methods. We consider that CD10, MUC2, and SIMA are detected in the intestinal phenotype, while HGM and ConA are detected in the gastric phenotype. The staining of CD10, MUC2, HGM, and ConA was carried out according to previous studies [5]. The staining of SIMA was carried out by the streptavidin-biotin (SAB) method with an antibody against SIMA (SIMA-4D3; NovoCastra, Newcastle, England) and SAB-PO (mouse) kits (Nichirei, Tokyo, Japan). Sections (4-μm-thick) were deparaffinized in xylene, hydrated through a graded series of ethanol, and immersed in 3% hydrogen peroxide, followed by immersion in 100% methanol for 30 min to inhibit endogenous peroxidase. To activate the antigens, the slides were placed in a microwave oven in 10 mM citrate buffer (pH 6.0) for 30 min. After a rinsing in phosphate-buffered saline (PBS), the slides were incubated in humid chambers with the primary antibodies, SIMA at 1:100, overnight at 4°C, followed by three washes with PBS. The sections were then incubated with biotinylated anti-mouse immunoglobulin (Ig) G, IgA, and IgM for 20 min, and with peroxidase-conjugated streptavidin for 20 min. After being washed in PBS, the slides were developed by being immersed in 0.01% H2O2 and 0.05% diaminobenzidine tetrahydrochloride (DAB) for 3 min. A light counterstaining with Mayer's hematoxylin was carried out. The results of staining were categorized into two groups: positive expression and negative expression. When more than 10% of the carcinoma cells in the neoplastic areas were stained, it was classified as positive expression. When fewer than 10% of the carcinoma cells in the neoplastic areas were stained, it was classified as negative expression.

Classification and comparisons of phenotypic expression

Classification of the phenotypes of the carcinomas and the background mucosas was based on the classification of intestinal metaplasia (complete-type intestinal metaplasia, incomplete-type intestinal metaplasia, or non-metaplastic gastric mucosa) by immunohistochemical staining, according to previous studies [5,18]. The phenotypes were classified into four groups; gastric type, incomplete-intestinal type, complete-intestinal type, and unclassified type, according to the combination of the expression of CD10, MUC2, SIMA, HGM, or ConA. Figure 1 summarizes the classification of the

<table>
<tr><td colspan="2"></td><td>HGM or ConA (+)</td><td>HGM and ConA (-)</td></tr>
<tr><td colspan="2">CD10(+)</td><td rowspan="2">Incomplete intestinal-type</td><td>Complete intestinal-type</td></tr>
<tr><td rowspan="2">CD10(-)</td><td>MUC2 or SIMA (+)</td><td></td></tr>
<tr><td>MUC2 and SIMA (-)</td><td>Gastric-type</td><td>Unclassified-type</td></tr>
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Fig. 1. Classification of phenotypes. The phenotypes were classified into four groups; gastric type, incomplete-intestinal type, complete-intestinal type, and unclassified type, according to the combination of the expression of CD10, MUC2, small-intestinal mucinous antigen (*SIMA*), human gastric mucin (*HGM*), or concanavalin A (*ConA*)

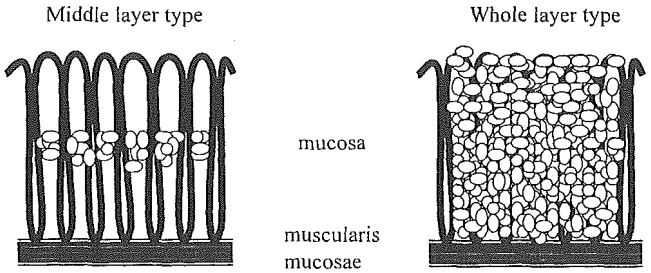

Fig. 2. Spreading features. The spreading features were classified into two patterns, the middle-layer type and the whole-layer type. In the middle-layer type, the carcinoma cells mainly spread in the gastric mucosa between the proper foveolae. In the whole-layer type, the carcinoma cells mainly expand in the gastric mucosa with destruction of the foveolae

Table 1. Clinicopathological findings

Carcinoma	Gastric type ($n = 20$)	Intestinal type ($n = 40$)	
Sex (male : female)	10:10	20:20	NS
Age (average, years)	54.4 ± 9.1	55.5 ± 11.5	NS
Location			NS
C	1	2	
M	12	22	
A	7	16	
Gross classification			NS
IIb	2	2	
IIc	18	37	
III	0	1	
Size (average, cm)	2.8 ± 1.7	3.5 ± 1.8	NS

phenotypes of the carcinomas and the background mucosas. We compared the clinicopathological findings and the morphological findings (including the existence of glandular structures or signet-ring cells and the spreading pattern) between gastric-type carcinomas and intestinal-type carcinomas (including incomplete-intestinal-type carcinomas and complete-intestinal-type carcinomas). The spreading patterns were classified into two types, middle-layer type and whole-layer type, as shown in Fig. 2.

Statistical analyses

The BMDP statistical package program (BMDP; Los Angeles, CA, USA) for an IBM (Armonk, NY, USA) 4381 mainframe computer was used for all analyses. The relationships between the clinicopathological findings or the morphological findings and the phenotypes were examined by the χ^2 test and Kruskal-Wallis test. The level of significance was less than 0.05.

Results

Clinicopathological findings

Gastric-type carcinoma is shown in Fig. 3. This carcinoma showed the middle-layer-type spreading pattern. The upper layer of the carcinoma was positive for HGM, while the lower layer of the carcinoma was positive for ConA, as was the normal layer structure of gastric proper mucosa. Incomplete-intestinal-type carcinoma is shown in Fig. 4. This carcinoma showed the whole-layer-type spreading pattern. This carcinoma was negative for HGM and ConA, and positive for SIMA. Complete-intestinal-type carcinoma is shown in Fig. 5. This carcinoma had structures of small trabeculae and microglands. This carcinoma was negative for HGM and ConA, while the laminar surface of the carcinoma was positive for CD10. The incidence of gastric-type carcinomas, incomplete intestinal-type carcinomas, and complete intestinal-type carcinomas was 33% (20 cases), 65% (39 cases), and 2% (1 cases), respectively.

Table 1 shows the clinicopathological findings of the 20 gastric-type carcinomas and 40 intestinal-type carcinomas (incomplete-intestinal-type carcinomas and complete-intestinal-type carcinomas). Intestinal-type carcinomas tended to be larger than gastric-type carcinomas, but there were no significant differences in any clinicopathological factors examined between gastric-type carcinomas and intestinal-type carcinomas.

Morphological findings

Table 2 shows the morphological findings of the 20 gastric-type carcinomas and the 40 intestinal-type carcinomas. Intestinal-type carcinomas had a glandular structure more frequently than gastric-type carcinomas. Gastric-type carcinomas had the middle-layer type spreading pattern more frequently than intestinal-type carcinomas. On the other hand, intestinal-type carcinomas had the whole-layer type spreading pattern more frequently than gastric-type carcinomas. In addition, the whole-layer-type carcinomas tended to be larger than the middle-layer-type carcinomas (average size, 3.3 cm vs 2.8 cm).

Relationship between carcinoma phenotype and that of the background mucosa

Table 3 shows the relationship between the phenotype of the carcinomas and that of the background mucosa. The phenotype of the carcinomas was significantly related to that of their own background mucosa ($P = 0.0440$). The spreading pattern of gastric-type carcino-

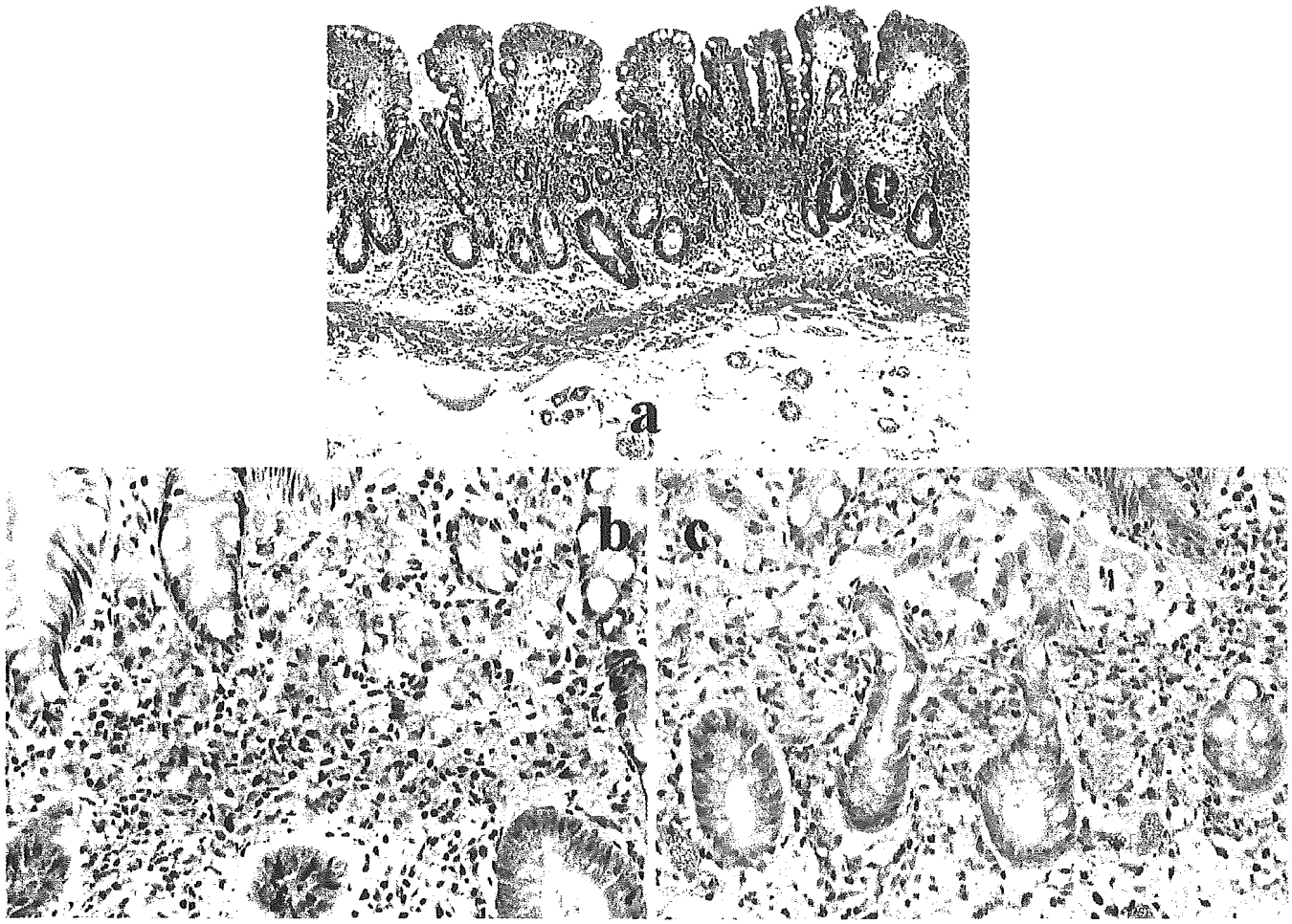

Fig. 3a–c. Gastric-type carcinoma. **a** This carcinoma shows middle-layer type spreading and arises in incomplete-intestinal-type background mucosa. ×50. **b** The upper layer of the carcinoma is positive for HGM. ×100. **c** The lower layer of the carcinoma is positive for ConA. ×100

Table 2. Morphological findings

Carcinoma	Gastric type (n = 20)	Intestinal type (n = 40)	
Glandular structure*			P = 0.0059
(−)	16	17	
(+)	4	23	
Signet-ring cell			P = 0.0641
(−)	5	20	
(+)	15	20	
Spreading pattern*			P < 0.0001
Middle-layer type	12	4	
Whole-layer type	8	36	

*Significant

mas tended to be the middle-layer type and the spreading pattern of intestinal-type carcinomas tended to be the whole-layer type, regardless of the phenotype of their own background mucosa.

Discussion

In this study, we classified the phenotypes of undifferentiated-type gastric carcinomas, based on the expression of CD10, MUC2, SIMA, HGM, or ConA

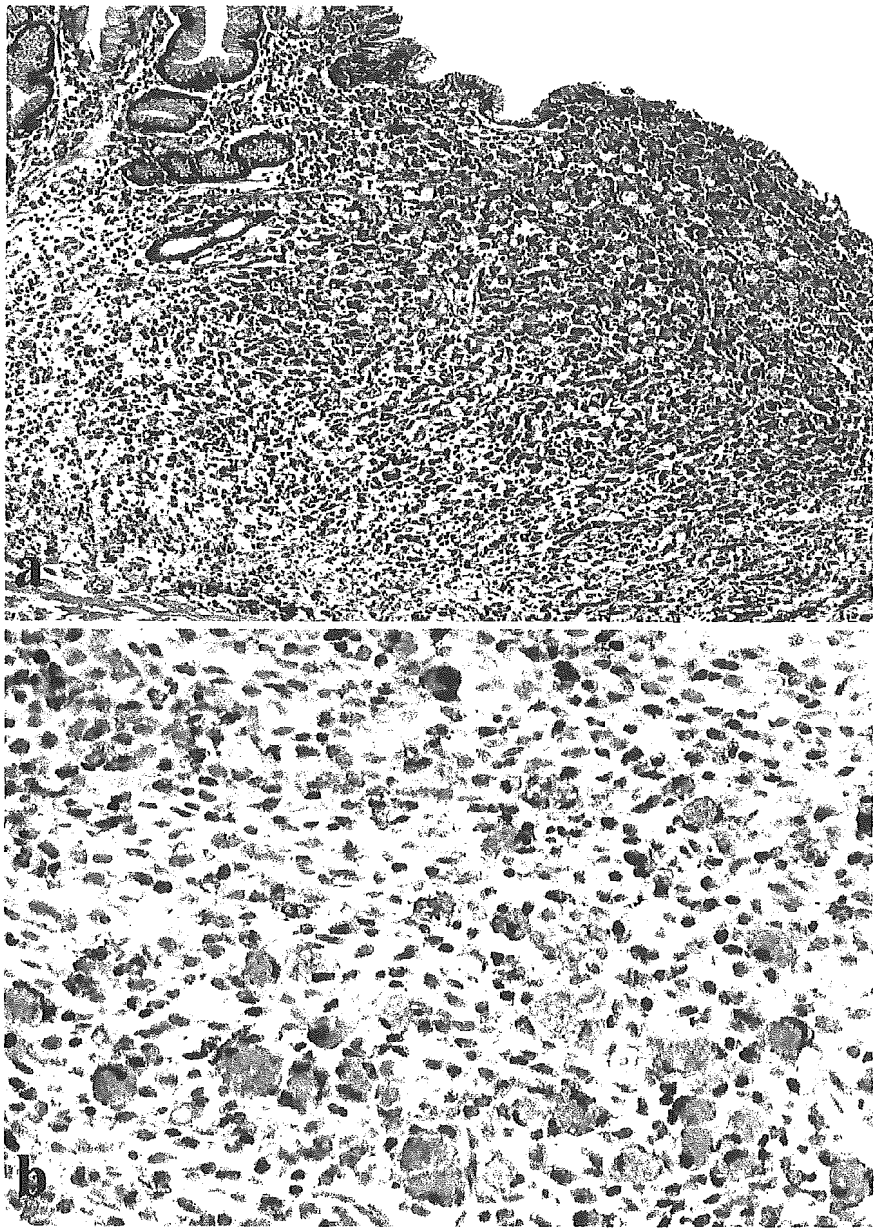

Fig. 4a,b. Incomplete-intestinal-type carcinoma. **a** This carcinoma shows whole-layer-type spreading pattern. ×50. **b** This carcinoma is positive for SIMA. ×100

Table 3. Relationship between the phenotype of the carcinomas and that of the background mucosa

Carcinoma	Gastric type (n = 20)	Intestinal type (n = 40)	
Background mucosa			P = 0.0440
Gastric type	13 (M, 8; W, 5)	15 (M, 2; W, 13)	
Intestinal type	7 (M, 4; W, 3)	25 (M, 2; W, 23)	

M, middle-layer-type carcinoma; w, whole-layer-type carcinoma

and the classification of intestinal metaplasia. In previous studies, it was shown that CD10, MUC2, HGM, and ConA were expressed in the brush border, the goblet cells, the gastric foveolar epithelium, and the pyloric glands, individually [19–26]. We employed SIMA, for the first time, for the evaluation of intestinal phenotypic expression. SIMA is a 1000-kDa mucin glycoprotein antigen that is known to be present in the goblet cells and the extracellular mucin of the small intestine. SIMA is not present in normal stomach and normal adult large

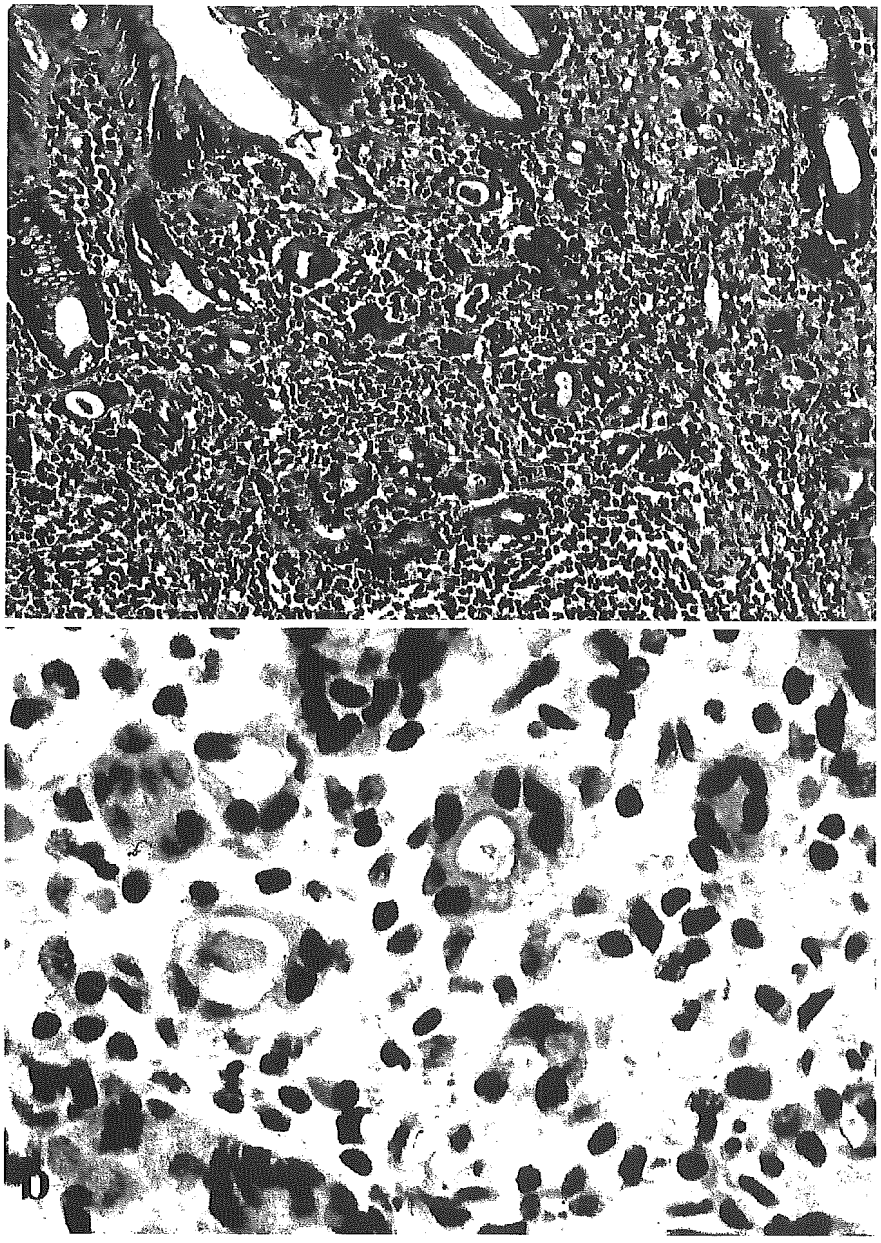

Fig. 5a,b. Complete-intestinal-type carcinoma. **a** This carcinoma has structures of small trabeculae and microglands. ×50. **b** The laminar surface of this carcinoma is positive for CD10. ×100

bowel. But SIMA is reported to be present in cancers of the stomach and large bowel [27–30]. In this study, 67% of undifferentiated-type gastric carcinomas had an intestinal phenotype. Sepulveda et al. [31] reported that 46% of undifferentiated-type gastric carcinomas had an intestinal phenotype. Fujimori et al. [32] reported diffuse-type gastric carcinomas that were composed of Paneth-cell-type carcinoma cells. Wang et al. [33] reported diffuse-type gastric carcinomas that expressed human colonic mucin (HCM14 or HCM21).

In differentiated-type gastric carcinomas, it was reported that intramucosal carcinomas tended to have a phenotype similar to the phenotype of the background mucosa. It has also been demonstrated that even intramucosal diffuse-type gastric carcinomas have the laminated structure of mucins similar to that in the background mucosa [34–35]. In the present study, several carcinomas were positive for the foveolar epithelial marker (HGM) in the upper layer and positive for the pyloric glandular marker (ConA) in the lower layer, similar to the layer structure of the gastric proper mucosa. Also, the phenotype of the carcinomas tended to imitate the phenotype of their own background mucosa, as reported in previous studies. It is considered that intramucosal carcinomas, even those of the undifferentiated type, tend to keep the phenotype of the background mucosa. Solcia et al. [36] reported that there were two carcinogenetic pathways of diffuse-type gastric carcinoma. We also consider that undifferentiated-type gastric carcinomas have a carcinogenetic pathway

not only from the gastric proper mucosa but also a carcinogenetic pathway from intestinal metaplasia. We considered that, as gastric carcinomas increased in size, the whole-layer type was shown, and that the progression of carcinomas was associated with a phenotypic shift from gastric-type expression to intestinal-type expression, as Yamachika et al. [15] and Yao et al. [16] have reported.

In this study, there were no clinicopathological differences between gastric-type carcinomas and intestinal-type carcinomas, but there were morphological differences. Gastric-type carcinomas more commonly had the middle-layer type, spreading pattern compared with intestinal-type carcinomas. In a carcinoma with the middle-layer type, spreading pattern the tumor margins tend to be unclear, because the carcinoma cells do not appear at the surface of the mucosa. So the margin of gastric-type carcinoma is considered to be unclear. Similarly, in differentiated-type carcinomas, Yoshino et al. [6] reported that the margin of gastric-type carcinoma was unclear compared with that in intestinal-type carcinoma. For gastric-type carcinomas, in particular the area to be resected and the choice of endoscopic mucosal resection (EMR) has to be carefully considered.

Our immunohistochemical method can be used to classify the phenotypes of gastric carcinomas, without regard to the presence or absence of a glandular structure, and we have shown that undifferentiated-type carcinomas frequently express an intestinal phenotype. There were differences in the growth patterns between undifferentiated-type gastric carcinomas with a gastric phenotype and those with the intestinal phenotype.

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