Neutrophil infiltration and the distribution of intestinal metaplasia is associated with metachronous gastric cancer following endoscopic submucosal dissection

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BACKGROUND: Endoscopic submucosal dissection (ESD) of early gastric cancer is a minimally invasive procedure. However, the risk for metachronous cancers after successful cancer treatment remains high and the risk factors for metachronous cancers have not been elucidated.

OBJECTIVE: To evaluate the risk factors for metachronous gastric cancers after ESD with a long-term follow-up.

METHODS: A total of 155 consecutive patients (119 men, 36 women, mean age 68.9 years) were treated with ESD between September 2000 and September 2009. Biopsy specimens were obtained from the greater curvature of the antrum and middle corpus to evaluate gastric mucosal status, including Helicobacter pylori, intestinal metaplasia (IM) and neutrophil infiltration (NI) before ESD. Follow-up endoscopy after ESD was scheduled at two and six months, one year and annually thereafter. H pylori eradication was recommended when possible.

RESULTS: The median follow-up period was 4.2 years. Metachronous gastric cancers were found in 23 of 155 patients (3.5% per year). No local recurrences were observed. The cumulative incidence of metachronous gastric cancer was significantly high in IM and NI in the corpus (P=0.0093 and P=0.0025, respectively [log-rank test]). The ORs for IM and NI in the corpus were 2.65 and 3.06, respectively, according to the Cox proportional hazards model (P=0.024 and P=0.0091, respectively).

CONCLUSIONS: The presence of IM and NI in the corpus was closely related to the development of metachronous gastric cancer after ESD.

Key Words: Endoscopic submucosal dissection; Helicobacter pylori; Intestinal metaplasia; Neutrophil infiltration; Stomach neoplasms
TABLE 1
Baseline characteristics of 155 patients

| Demographic characteristics                        | Total | Metachronous (n=23) | No recurrence (n=132) | P   |
|---------------------------------------------------|-------|---------------------|-----------------------|-----|
| Sex, male/female, n                               | 119/36| 20/3                | 99/33                 | 0.19|
| Age, years, mean (range)                          | 68.9 (50–83) | 69.2 (57–80) | 68.8 (50–83) | 0.84|
| Follow-up period, years (range)                   | 4.23 (1.02–10.6) | 4.11 (1.05–7.35) | 4.25 (1.02–10.8) | 0.78|
| Outcome (alive/dead)                              | 149/6 | 23/0                | 126/6                 | 0.16|
| Helicobacter pylori status after endoscopic submucosal dissection, n (%) |       |                     |                       | 0.17|
| Negative                                          | 25 (16.1) | 1 (4.3)            | 24 (18.1)             |     |
| Eradication                                       | 100 (64.5) | 17 (74.0)          | 83 (62.9)             |     |
| Persistent                                        | 30 (19.4) | 5 (21.7)           | 25 (19.0)             |     |
| Histological characteristics, n (%)              |       |                     |                       |     |
| Intestinal metaplasia in the antrum               | 101 (65.2) | 15 (65.2)          | 86 (65.2)             | 0.99|
| Intestinal metaplasia in the corpus               | 74 (47.7) | 15 (65.2)          | 59 (44.7)             | 0.068|
| Neutrophil infiltration in the antrum             | 30 (19.4) | 8 (34.8)           | 22 (16.7)             | 0.056|
| Neutrophil infiltration in the corpus             | 50 (32.3) | 15 (65.2)          | 35 (26.5)             | 0.0004|
| Pepsinogen, ng/mL, mean (n=127)                   |       |                     |                       |     |
| I                                                 | 44.1 | 27.2               | 47.2                  | 0.17|
| II                                                | 16.8 | 13.3               | 17.5                  | 0.24|
| III                                               | 2.61 | 2.10               | 2.71                  | 0.15|
| Tumour characteristics                            |       |                     |                       |     |
| Location, upper/middle/lower, n                   | 14/65/76 | 1/13/9            | 13/52/67              | 0.27|
| Size, mm, mean                                    | 15.7 | 20.3               | 14.9                  | 0.0048|
| Macroscopic type, protruded/depressed, n          | 88/67 | 12/11              | 76/56                 | 0.63|
| Pathology, adenoma/cancer, n                      | 31/124 | 5/18               | 26/106                | 0.82|

Patients
A total of 201 consecutive patients were treated using ESD at the University of Tokyo Hospital (Tokyo, Japan) between September 2000 and September 2009. All baseline data and endoscopic findings obtained before ESD were evaluated retrospectively from patient records. The location, macroscopic types and histological findings of the tumours were categorized according to the Japanese classification of gastric carcinoma (23). Expanded ESD criteria have been proposed by Gotoda et al (24). All patients were followed up for at least one year. Forty-six patients were excluded from the study; 19 did not meet the ESD specimen histology criteria and required additional surgery, five had remnant stomach cancers, nine were followed for up to one year, four did not have H pylori information and, in nine patients, ESD was performed before enrollment. The present study was approved by the Institutional Review Board of the University of Tokyo.

Disease assessment
Experienced endoscopists performed the gastrointestinal endoscopy procedures, and gastric cancer was diagnosed by histology. Biopsy specimens were obtained from the greater curvature of the antrum and middle corpus to evaluate gastric mucosal status including H pylori, IM and NI before ESD (22). Both a rapid urease test (Helicocheck, Otsuka Pharmaceuticals, Japan) and histopathological examination were performed. A positive result on at least one test was deemed to be evidence of H pylori infection. Blood samples were also collected before ESD to measure serum levels of pepsinogen I and II using ELISA kits (LS test Eiken Kagaku, Inc, Japan). ESD was performed as described previously (8,9). Follow-up endoscopy after ESD for detection of metachronous gastric cancers was scheduled at two and six months, one year and annually thereafter. Lesions detected within one year of initial ESD were regarded as synchronous multiple lesions because microcancers may have been missed at the time of ESD (12,25). Metachronous gastric cancers were defined as new gastric cancers in different areas from the initial lesion and occurring at least one year after the initial ESD. Eradication of H pylori was recommended when possible. Metachronous gastric cancer was defined as the development of a new carcinoma in areas other than the primary gastric cancer site at least one year after ESD. H pylori-positive patients were treated with triple therapy consisting of 200 mg clarithromycin, 750 mg amoxicillin and 30 mg lansoprazole twice daily for one week after ER. Patients in whom H pylori was not eradicated were treated with second-line therapy consisting of 250 mg metronidazole instead of clarithromycin (26). Eradication was confirmed by a negative 13C-urea breath test (8,9).

Statistical analyses
Study subjects were categorized according to the presence of the metachronous gastric cancer. All analyses were performed using JMP version 9 (SAS Institute, USA). Student’s t tests were used for intergroup comparison of mean age, follow-up period and tumour size. The other patient clinical characteristics and histopathological characteristics of the gastric mucosa and cancers were compared using the χ² test or Fisher’s exact test, as appropriate. Relative risks for metachronous gastric cancer were calculated using the Cox proportional hazards model. Differences were considered to be statistically significant at the 5% probability level.

RESULTS
Baseline characteristics of the study subjects
Baseline clinical characteristics of the patients are summarized in Table 1. A total of 155 patients (119 men, 36 women, mean age 68.9 years) were followed for up to 10.8 years (mean 4.2 years). Six patients died during the study period, but none of the deaths were related to gastric cancer. Twenty-three (14%) patients developed metachronous gastric cancers (3.5% per year).
Metachronous gastric cancer risk following ESD

Of the 155 patients, 25 were *Helicobacter pylori* negative, 27 had *H pylori* eradicated before ESD, 73 received eradication therapy and 30 experienced continuing *H pylori* infection. IM at the greater curvature of the antrum and middle corpus was observed in 101 (65.1%) and 74 (47.7%) patients, respectively. NI was observed in 30 (19.4%) and 50 (32.3%) patients, respectively.

Factors related to metachronous gastric cancers

The cumulative incidence of metachronous gastric cancer is summarized in Table 2, and Figures 1 and 2. The ORs for metachronous gastric cancers in IM and NI in the corpus were 3.00 (P=0.010) and 3.47 (P=0.0034). IM and NI in the corpus and tumour size were risk factors. Successful *H pylori* eradication did not contribute significantly to the reduction of risk for

| Table 2 | Cumulative incidence of metachronous gastric cancer, univariate analysis* |
|-------------------------|-----------------------------|-------|
| **OR (95% CI) P**       | **Demographic characteristics** | **Histological characteristics** |
| Sex                      | 1.78 (0.61–7.55) 0.32         | **Intestinal metaplasia in the antrum** 0.99 (0.43–2.46) 0.97 |
| Age                      | 1.02 (0.96–1.08) 0.60         | **Intestinal metaplasia in the corpus** 3.00 (1.29–7.51) 0.010 |
| *Helicobacter pylori* status after ESD | 0.64 | **Neutrophil infiltration in the antrum** 1.89 (0.76–4.39) 0.16 |
| Negative                 | 1                           | **Neutrophil infiltration in the corpus** 3.47 (1.51–8.63) 0.0034 |
| Eradication              | 2.56 (0.52–46.3) 0.30         | **Pepsinogen (measured in 127 patients)** |
| Persistent               | 2.50 (0.40–48.3) 0.36         | I 0.98 (0.96–1.01) 0.11 |
|                          |                             | II 0.96 (0.92–1.02) 0.18 |
|                          |                             | III 0.83 (0.59–1.17) 0.28 |
| **Histological characteristics** | **Histological characteristics** | **Histological characteristics** |
| **Location**             | **Location**                 | **Location** |
| Upper                    | 1                           | Upper 1 |
| Middle                   | 2.26 (0.45–41.2) 0.38         | Middle 2.26 (0.45–41.2) 0.38 |
| Lower                    | 1.21 (0.23–22.4) 0.85         | Lower 1.21 (0.23–22.4) 0.85 |
| **Tumour size**          | **Tumour size**              | **Tumour size** |
|                          | 1.05 (1.01–1.09) 0.021        | Upper 1.05 (1.01–1.09) 0.021 |
|                          |                              | Middle 2.26 (0.45–41.2) 0.38 |
|                          |                              | Lower 1.21 (0.23–22.4) 0.85 |
| **Macroscopic type (protruded/depressed)** | **Macroscopic type (protruded/depressed)** | **Macroscopic type (protruded/depressed)** |
|                          | 0.79 (0.35–1.83) 0.58         | **Pathology (cancer/adenoma)** 1.18 (0.47–3.59) 0.73 |
| **Pathology (cancer/adenoma)** | **Pathology (cancer/adenoma)** | **Pathology (cancer/adenoma)** |
|                          |                             | Upper 1 |
|                          |                             | Middle 2.26 (0.45–41.2) 0.38 |
|                          |                             | Lower 1.21 (0.23–22.4) 0.85 |
| *Cox proportional hazards model. ESD Endoscopic submucosal dissection*
metachronous gastric cancer in the present analysis (OR 2.56 [95% CI 0.52 to 4.63]; P=0.30).

**Multivariate analysis for metachronous gastric cancer**

The multivariate analysis of tumour size, IM and NI in the corpus showed that IM and NI in the corpus were independent risk factors for metachronous gastric cancer (Table 3). The ORs for metachronous gastric cancer were 2.65 (95% CI 1.13 to 6.66; P=0.024) in the IM-positive group in the corpus and 3.06 (95% CI 1.32 to 7.64; P=0.009) in the NI-positive group in the corpus.

**DISCUSSION**

The results of the present single-centre study involving 155 consecutive patients showed that IM and NI in the corpus were related to metachronous gastric cancer. IM and NI were high-risk microenvironments and risk factors for primary and metachronous gastric cancers. Not only H pylori eradication but also annual follow-up endoscopy may be important for improving the prognosis of patients with metachronous gastric cancer.

Although many studies of residual gastric cancer have been conducted (27,28), few reports of metachronous gastric cancers after ESD for early gastric cancer are available. The rate of recurrence of early gastric cancer in the gastric stump has been estimated to be 1% to 3% (13,29). An increased recurrence rate would be expected in patients treated with ESD, in proportion to the larger area of gastric mucosa remaining in these patients. Other studies have reported that the annual incidence of metachronous gastric cancer after EMR is 2.5% to 4% (12,22). In the present study, the annual incidence was approximately 3.5%.

In their randomized controlled study, Fukase et al (20) reported that eradicating H pylori reduced the risk for developing metachronous gastric cancer in patients treated with EMR. In our previous study, we reported that IM, NI and the gastritis pattern are related to developing gastric cancer. All patients with metachronous gastric cancer were treated completely with ESD or surgery. Annual endoscopy follow-up may be useful for detection of metachronous gastric cancer during the early stage and prevention of gastric cancer death.

A limitation of our study may be a retrospective cohort study in consecutive patients. A prospective study is needed to determine whether baseline histological characteristics can be predictive factors for metachronous gastric cancer and be used to determine the intensity of surveillance endoscopy among this patient population.

**CONCLUSION**

The presence of IM and NI in the corpus was closely related to the development of metachronous gastric cancer after ESD.

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