Clinical and Histopathological Features of Early Gastric Cancer With Unclear Lateral Demarcation

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Abstract

Background

Magnifying-endoscopy with narrow band imaging (M-NBI) is useful to determine lateral demarcation of early gastric cancers, but determining the lateral demarcation is sometimes difficult. Features related to the unclear lateral demarcation remain unknown. We evaluated the clinical and histopathological features of early gastric cancers with unclear lateral demarcation by M-NBI.

Methods

This single-center retrospective cohort study analyzed early gastric cancer treated by endoscopic submucosal dissection (ESD) from January 2013 to August 2015. We evaluated clinicopathological and immunohistochemical features using anti-p53, -Ki-67, -MUC5AC, -MUC6, -MUC2, and -CD10 antibody staining. We compared the lateral demarcation between the demarcation clear (DC) and demarcation unclear (DU) lesions by using M-NBI.

Results

A total of 224 differentiated adenocarcinomas (DU group: 18 lesions; DC group: 206 lesions) were analyzed. The history of successful *Helicobacter pylori* eradication was significantly more frequent in the DU group ($p = 0.001$). We examined tissues of 72 lesions immunohistochemically, including 18 lesions in the DU group and 54 randomly selected lesions in the DC group. Non-neoplastic superficial epithelium is more frequently observed in the DU group ($p = 0.0058$). Additionally, the DU group showed a significantly higher expression of gastric phenotype marker ($p = 0.023$), lower p53 score ($p = 0.0002$), and lower Ki-67 labeling index ($p = 0.0293$). The non-neoplastic superficial epithelium and low p53 score were significant independent variables associated with unclear lateral demarcation by M-NBI in the multivariate analysis.

Conclusions

Non-neoplastic superficial epithelium and low p53 score were associated with the difficultly in determining lateral demarcation in early gastric cancers by M-NBI.

Introduction

Various modalities have been developed for the pre-procedural diagnosis of lateral demarcation of early gastric cancers. In particular, magnifying-endoscopy with narrow band imaging (M-NBI) is useful for pre-procedural diagnosis, because it provides good visualization for both superficial mucosal structure and blood vessel architecture.[1–3] The clear determination of its lateral demarcation is very important for a complete *en-bloc* resection by endoscopic submucosal dissection (ESD), but poor determination can

sometimes occur. It is well known that undifferentiated adenocarcinomas show unclear lateral demarcation compared to differentiated adenocarcinomas.[4, 5] We sometimes misdiagnose the lateral demarcation even in differentiated adenocarcinomas. Recently, the *Helicobacter pylori* eradication therapy has spread worldwide; therefore, early gastric cancers are sometimes detected after successful eradication.[6, 7] Some reports show that early gastric cancers that developed after eradication therapy sometimes show an unclear lateral demarcation.[8] Regarding their features, they are reported to have a normal columnar epithelium on the surface of the tumor, a mucin phenotype marker of gastric or gastrointestinal type, and a low Ki-67 labeling index (LI). However, any features related to the unclear lateral demarcation remain unknown. The aim of this study was to evaluate the clinicopathological and immunohistochemical features of differentiated adenocarcinomas with unclear lateral demarcation by M-NBI.

Methods

Study design

We conducted a retrospective cohort study of early gastric cancers treated by ESD from January 2013 to August 2015 in our institution. Well or moderately differentiated adenocarcinomas diagnosed by two pathologists during the period were examined. Adenomas, undifferentiated or undifferentiated-mixed cancers, and *H. pylori*-unrelated gastric cancers, such as fundic gland type cancers, were excluded. All study participants provided written informed consent, and the study was approved by the institutional review board (IRB) and ethics comittee of Osaka university hospital (IRB number: 16451). No patients were under 20 years old. This study was conducted in accordance with the principles laid down in the Helsinki Declaration.

Tumor classification

We performed the endoscopic diagnosis according to the vessel plus surface classification system by M-NBI.[3] The demarcation line (DL) separates a lesion from the normal surrounding mucosal area. If DL was present all around the lesion, we classified the lesion into the demarcation clear (DC) group. If DL was absent for a part of the lesion, we categorized the lesion into to the demarcation unclear (DU) group. All lesions with still images were evaluated at a conference attended by more than six endoscopic experts and divided into two groups (DC and DU).

Clinical examination

We analyzed the differences in clinical features of early gastric cancers, including size, location, macroscopic type, color, and history of *H. pylori* eradication, between the two groups. We defined successful *H. pylori* eradication therapy, if the patients had a history of *H. pylori* eradication therapy and received at least one negative test of the following methods: tests for the presence of serum IgG antibodies against *H. pylori*, the ^{13}C urea breath test, and stool test for *H. pylori* antigen. We evaluated the presence of *H. pylori* at the time of lesion detection. In addition, we compared the short-term outcome of

treatment, including the rate of *en-bloc* or curative resection, procedure time, the rate of complication, such as perforation and delayed bleeding, and the rate of additional surgical resection between the two groups. *En-bloc* resection was defined as one-piece resection endoscopically, whereas curative resection was defined as *en-bloc* resection histopathologically with the absence of lymphovascular invasion and presence of tumor negative lateral and vertical margins of the resected specimens according to the guideline of the indication criteria for curative resection.[9] Perforation was defined as a defect of the muscular layer during ESD or presence of free air detected by computed tomography after ESD. Delayed bleeding was defined as hematochezia after ESD that required endoscopic hemostasis.[10]

Histological examination

Formalin-fixed and paraffin-embedded specimens from the included lesions were retrieved. All hematoxylin and eosin (HE)-stained sections were evaluated by two pathologists. Histological type, combined ulcer or ulcer scar, and depth of invasion were evaluated. Additionally, we evaluated the histopathological features on the superficial epithelium and divided them into the following three categories: non-neoplastic superficial epithelium, partial non-neoplastic superficial epithelium, and neoplastic superficial epithelium. Partial non-neoplastic superficial epithelium was defined as the presence of both non-neoplastic and neoplastic epithelia.

Evaluation of histopathological structure

The randomly selected visual field (100 ·) in both the lesion and the background mucosa from one tissue was assessed. One of the authors evaluated the width of the interfoveolare, length of duct, and width of pit to compare the difference in the histopathological structure between the lesion and the surrounding mucosa in both groups.

Immunohistological staining

The tissue sections cut at 4-μm thickness were used. The immunohistochemical staining for p53 (DO-7, 1:250 dilution; DAKO, Glostrup, Denmark), Ki-67 (MIB-1, 1:1000 dilution; DAKO), MUC5AC (CLH2, 1:50 dilution; DAKO), MUC6 (CLH5, 1:50 dilution; Santa Cruz Biotechnology, Dallas, TX), MUC2 (CCP58, 1:50 dilution; DAKO), and CD10 (56C6, 1:40 dilution; DAKO) antibody-antigen complexes were detected using a VECTASTAIN ABC kit (mouse; Vector Laboratories, Burlingame, CA) according to the manufacturer's protocol.[11] A negative control was designed by using phosphate-buffered saline instead of primary antibodies. Slices were counterstained with hematoxylin, dehydrated, and mounted. Finally, stained sections were evaluated using light microscopy.

Evaluation of immunohistochemical staining

We selected one tissue section with the largest cancerous area for each resected lesion. The randomly selected visual field (100·) in each lesion was assessed and scored. A brown stain was regarded as positive. The expressions of p53 and Ki-67 were quantified using image processing software (WinROOF, ver.5.7.2; Mitani Corp., Tokyo, Japan). This software measures the color intensity and transforms the color selected into a percentage in each field. We calculated the intensity of staining at the threshold of

color and the extension of staining at the percentage of positive cells against total cancer cells. The p53 immunostaining was analyzed semi-quantitatively using a scoring system for both intensity (0, no staining; 1, weak staining; 2, moderate staining; 3, strong staining) and extension (0, no unclear staining; 1, < 10% staining; 2, 10%-50% staining; 3, > 50% staining). The score for intensity and extension was summed for each lesion. We classified a score of > 4 as marked staining for p53 according to a previous report.[12] Ki-67 LI was calculated as the percentage of positive cells against total cancer cells. We classified the lesions into a high group if Ki-67 LI was greater than the median value, which was also according to a previous report.[13]

The mucin expression of tumor cells was examined with gastric phenotype markers, such as MUC5AC and MUC6, and intestinal phenotype markers, such as MUC2 and CD10. The results of each phenotype marker were defined as positive if > 10% of the tumor cells were stained. Tumors were classified by phenotype as gastric (G type), intestinal (I type), gastrointestinal (GI type), and null (N type); G type tumors expressed only a gastric phenotype marker, whereas I type tumors expressed only an intestinal phenotype marker. GI type tumors expressed both gastric and intestinal phenotype markers, whereas N type tumors were negative for all markers.[14] We evaluated the expression of each mucin phenotype marker not only in the tumor but also in the surrounding mucosa.

Statistical methods

Categorical variables were presented as frequencies and proportions. They were compared between the two groups by using Fisher's exact test. Continuous variables were presented as medians (interquartile range [IQR]). They were compared between the two groups by using Wilcoxon rank sum test. A p-value < 0.05 was considered significant. To reveal the factors associated with unclear lateral demarcation by M-NBI, univariate and multivariate logistic regression analyses were performed. All statistical analyses were performed with JMP statistical software ver. 14 (SAS Institute Inc, Cary, NC).

Results

Clinicopathological features

A total of 265 tumors were treated by ESD from January 2013 to August 2015 at our institution. Among them, 41 lesions, including 15 adenomas, 22 undifferentiated or mixed cancers and four *H. pylori*-unrelated gastric cancers, including three fundic gland type and one pyloric gland type lesion, were excluded. A total of 224 differentiated adenocarcinomas were analyzed in this study. As a result of the evaluation, 18 and 206 lesions were divided into the DU and DC groups, respectively (Fig. 1).

We compared the clinical characteristics between the DU and DC groups (Table 1). There were no differences in size, location, macroscopic type, color, histological type, combined ulcer or ulcer scar, and depth of invasion. The history of successful *H. pylori* eradication was significantly more frequent in the DU group than in the DC group (p = 0.001). In the short-term clinical outcome of the treatment, the *en-bloc* resection rate was lower in the DU group than in the DC group because of the positive horizontal margin

(p = 0.033). There were no differences in the procedure time and complication rate between the two groups.

Table 1
Clinicopathological features of the study subjects

	DU group	DC group	p value
Lesions, n	18	206	
Median size, mm [quartile]	9 [5.3, 20]	13 [8, 20]	n.s. [1]
Location (U/M/L), n (%)	1 (5)/ 5 (28)/ 12 (67)	11 (5)/ 91 (45)/ 104 (50)	n.s. [2]
Macroscopic type, n (%)	6 (33)	107 (51)	n.s.[2]
Elevated, 0-I, IIa	12 (67)	99 (49)	
Depressed, 0-IIb, IIc			
Color, n (%)	5 (28)	73 (36)	n.s.[2]
Red	13 (72)	133 (64)	
Isochromatic or white			
H. pylori **status, n (%)**	3 (17)	64 (31)	**0.001**[2]
Positive			
Post-successful H. pylori **eradication**	12 (66)	53 (26)	
Negative	3 (17)	89 (43)	
Histological type, n (%)	14 (78)	186 (90)	n.s. [2]
tub1	4 (22)	20 (10)	
tub2			
Combined ulcer or ulcer scar, n (%)	4 (22)	23 (11)	n.s. [2]
Depth of invasion, n (%)	16 (89)	181 (88)	n.s.[2]
M	2 (11)	25 (12)	
SM			

[1]Wilcoxon rank sum test, [2]Fisher's exact test

Abbreviations: n.s, not significant; U, M, and L, upper, middle, lower third of the stomach; tub1 and tub2, well and moderately differentiated tubular adenocarcinoma; M and SM, mucosa and submucosa; DU, demarcation unclear; DC, demarcation clear

Evaluation of histopathological structures between the cancerous lesion and the surrounding mucosa

To reveal the cause of unclear lateral demarcation of early gastric cancers in the DU group, we evaluated the histopathological structures between the cancerous lesion and the surrounding mucosa. The DU group had no differences in width of interfoveolare ($p = 0.987$), length of duct ($p = 0.351$), and width of pit ($p = 0.296$) between the lesion and the surrounding mucosa, whereas the DC group had significant differences in width of interfoveolare ($p < 0.01$), length of duct ($p < 0.01$), and width of pit ($p = 0.018$) between them (Fig. 2a-c).

Evaluation of histopathological features on superficial epithelium

We considered that a history of successful *H. pylori* eradication might influence the unclear lateral demarcation by M-NBI. We randomly selected 36 lesions with a history of successful *H. pylori* eradication and 18 lesions without such history in the DC group with reference to the frequency of a history of successful *H. pylori* eradication in the DU group. A total of 72 lesions, including 18 lesions in the DU group and 54 lesions in the DC group, underwent histopathological and immunohistochemical examinations.

Then, we evaluated the features of the superficial epithelium on the cancerous lesions between the DU and DC groups. Representative images of the partial and non-neoplastic superficial epithelia are shown in Fig. 3a. The DU group was more likely to have a non-neoplastic superficial epithelium than the DC group ($p = 0.0058$) (Fig. 3b). There was no difference in the frequency of partial non-neoplastic superficial epithelium between the two groups.

Evaluation of mucin phenotype

Next, we performed the immunohistochemical staining regarding the mucin phenotype marker between the DU and DC groups (Fig. 4a). We classified the mucin phenotype marker into the following two groups: gastric phenotype marker, including G and GI type, and non-gastric phenotype marker, including I and N type. The DU group had a higher expression of gastric phenotype marker in the cancerous lesion than the DC group ($p = 0.023$) (Fig. 4b). In the surrounding mucosa, there was no significant difference in the mucin phenotype marker between the two groups.

Evaluation of p53 positivity

We performed p53 staining to evaluate the difference in the oncogenic potential. The proportion of the lesions with low p53 score was significantly higher in the DU group than in the DC group ($p = 0.0002$) (Fig. 5a, b).

Evaluation of proliferation activity

We examined the Ki-67 LI to evaluate the difference in the proliferation activity between the two groups. The proportion of the lesions with low Ki-67 LI (p = 0.0293) was higher in the DU group than in the DC group (Fig. 5c, d).

Factors associated with unclear lateral demarcation by M-NBI

Multivariate analysis revealed that the presence of non-neoplastic superficial epithelium and low p53 score were independent variables associated with unclear lateral demarcation by M-NBI (Table 2).

Table 2
Factors associated with unclear lateral demarcation by magnifying endoscopy with narrow-band imaging

	Univariate OR (95% CI)	p value	Multivariate OR (95% CI)	p value
Presence of non-neoplastic superficial epithelium	4.89 (1.56–15.30)	0.007	9.49 (2.13–57.8)	0.003
Presence of gastric phenotype marker	5.50 (1.15–26.36)	0.023	3.12 (0.59–25.7)	0.189
Low p53 score	19.72 (2.45–158.9)	< 0.001	29.52 (3.99–671.4)	< 0.001
Low Ki-67 LI	3.78 (1.18–12.13)	0.029	2.57 (0.64–11.7)	0.185
Abbreviations: n.s, not significant; OR, odds ratio; LI, labeling index				

Discussion

In this study, we examined the clinicopathological and immunohistochemical features contributing to the unclear lateral demarcation of early gastric cancers by M-NBI. Yao *et al.* reported that a lesion without DL could be diagnosed as non-cancer.[15] However, we sometimes encounter cases of early gastric cancer with unclear DL. In fact, our study demonstrated that cancers without DL was found in 18 lesions out of 224 lesions (8%) in which the diagnosis of cancer was made by two pathologists who confirmed the diagnosis by immunohistochemical staining using anti-p53 and -Ki-67 antibodies. The lesions without DL are sometimes caused by the covering tumor cells showing a horizontal extension in the deep layers of the mucosal lamina propria by the superficial epithelium. Thus, recognizing the lateral demarcation by M-NBI have limitations. Ito *et al.* reported that the epithelium with low-grade atypia appeared on the surface of gastric cancer after *H. pylori* eradication.[16] Kobayashi *et al.* explained this phenomenon as a repair process of the erosion on the surface of cancers [17], whereas Saka *et al.* considered this epithelium as an existing crypt epithelium that avoided cancer replacement.[18] The pathogenesis of non-neoplastic superficial epithelium is still poorly understood. Our data show that non-neoplastic superficial epithelium often present with a crypt epithelium and MUC5AC positivity, and goblet cells with MUC2 positivity in the

mucin phenotype marker rarely appeared. In fact, there were no tumor cells on the superficial surface, and non-neoplastic epithelium with a gastric phenotype marker was replaced. Therefore, we considered this phenomenon was a regeneration after anti-inflammation.

The non-neoplastic superficial epithelium, gastric phenotype marker, low p53 score, and low Ki-67 LI were significant factors associated with unclear lateral demarcation by M-NBI in our histopathological and immunohistochemical analyses. In particular, non-neoplastic superficial epithelium and low p53 score were found to be independent and significant factors in the multivariate logistic regression analyses. We considered that these factors interfere in obtaining accurate endoscopic findings. We considered that the DU group has a small difference in the histological feature between the lesion and the surrounding mucosa because of the wide interfoveolare, long tumor duct, and open pit. We presumed that these findings are due to the fact that there are few tumor cells on the superficial surface, and tumor cells develop in the deep layers of the mucosal lamina propria.

In our study, 72 lesions, including 18 lesions in the DU group and 54 lesions in the DC group, underwent immunohistochemical examinations. The number of lesions in the DU group was small; thus, we randomly selected 54 lesions in the DC group with reference to the frequency of the history of successful *H. pylori* eradication in the DU group. We could not retrospectively evaluate the reason for undergoing *H. pylori* eradication; thus, we selected the objective lesions using a random number list, instead of using a propensity score.

The limitations of this study were its retrospective and single-center design and the small number of lesions in the DU group.

Conclusions

In conclusion, our study demonstrated that unclear lateral demarcation by M-NBI occurs when the tumor cells developed in the deep layers of the mucosal lamina propria. In addition, these lesions are characterized by the presence of a gastric phenotype marker, low p53 score, and low Ki-67 LI. Non-neoplastic superficial epithelium and low p53 score were associated with the difficultly in determining lateral demarcation in early gastric cancers by M-NBI. The evaluation of clinicopathological and immunohistochemical features associated with unclear lateral demarcation can facilitate the performance of appropriate treatment for these lesions.

List Of Abbreviations

M-NBI: Magnifying-endoscopy with narrow band imaging; ESD: Endoscopic submucosal dissection; DC: Demarcation clear; DU: Demarcation unclear; LI: labeling index; DL: Demarcation line; HE: Hematoxylin and eosin; G: gastric; I: intestinal; GI: gastrointestinal; N: null;

Declarations

Ethics approval and consent to participate

All study participants provided written informed consent, and the study was approved by the institutional review board (IRB) and ethics comittee of Osaka university hospital (IRB number: 16451). No patients were under 20 years old. This study was conducted in accordance with the principles laid down in the Helsinki Declaration.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable.

Authors' contributions

KN and YH analyzed and interpreted the patient data, and were major contributors in writing the manuscript. RU, TI, and KK analyzed and interpreted the data and helped to draft the manuscript. AS, SY, and YT conceived of the idea of the study, and analyzed and interpreted the data. SS, HI, and TT contributed to the design of the study, and analyzed and interpreted the data. All authors read and approved the final manuscript.

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Figures

Figure 1

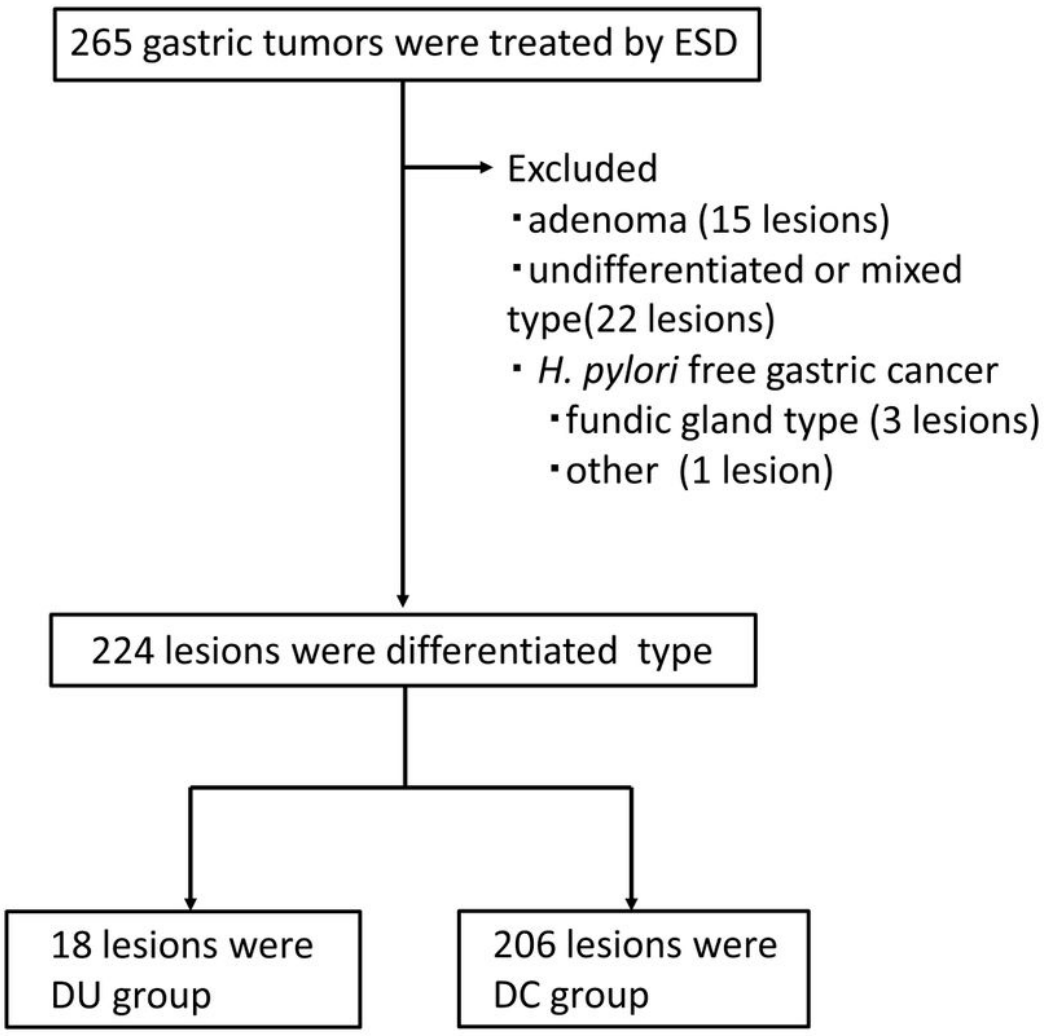


Figure 1

Study flow chart for early gastric cancers treated by endoscopic submucosal dissection.

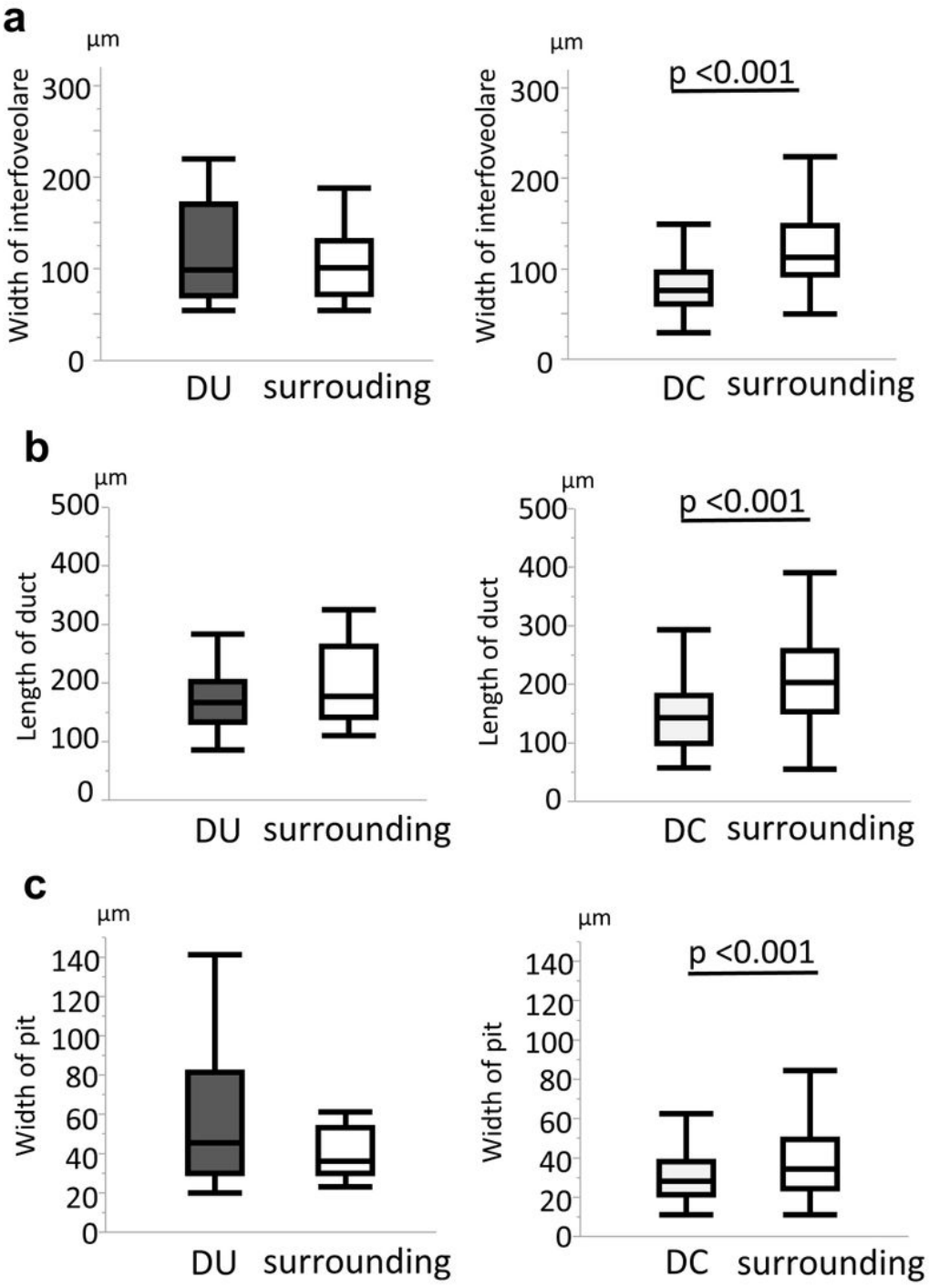
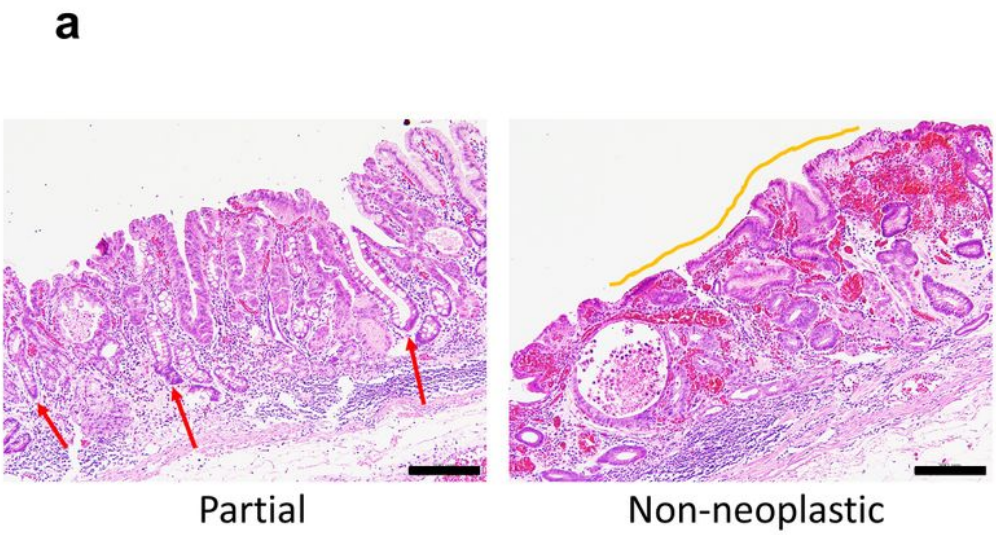


Figure 2

Difference in the histopathological structures between the cancerous lesion and the surrounding mucosa. a. Comparison of interfoveolare width between the cancerous lesion and the surrounding mucosa in the DU and DC groups. b. Comparison of duct length between the cancerous lesion and the surrounding mucosa in the DU and DC groups. c. Comparison of width of pit between the cancerous lesion and the surrounding mucosa in the DU and DC groups. DU, demarcation unclear; DC, demarcation clear

Figure 3



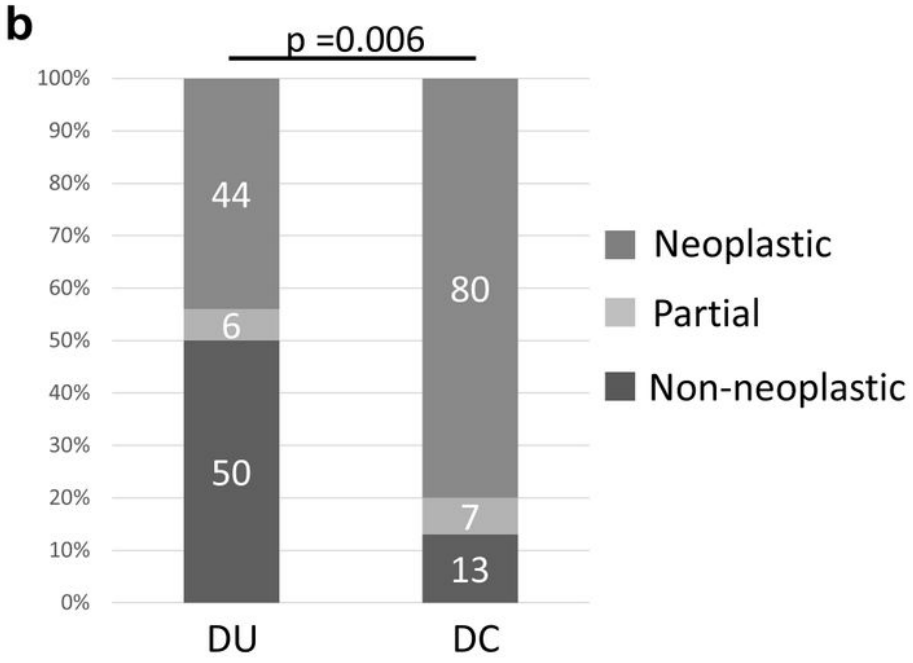


Figure 3

Difference in the features of superficial epithelium of the tumor between the DU and DC groups. a. Representative images of partial and non-neoplastic superficial epithelia are shown. The red arrow shows a non-tumor cell. The yellow line shows a non-tumor cell on the surface. b. Comparison of the superficial epithelium between the DU and DC groups. DU, demarcation unclear; DC, demarcation clear

Figure 4

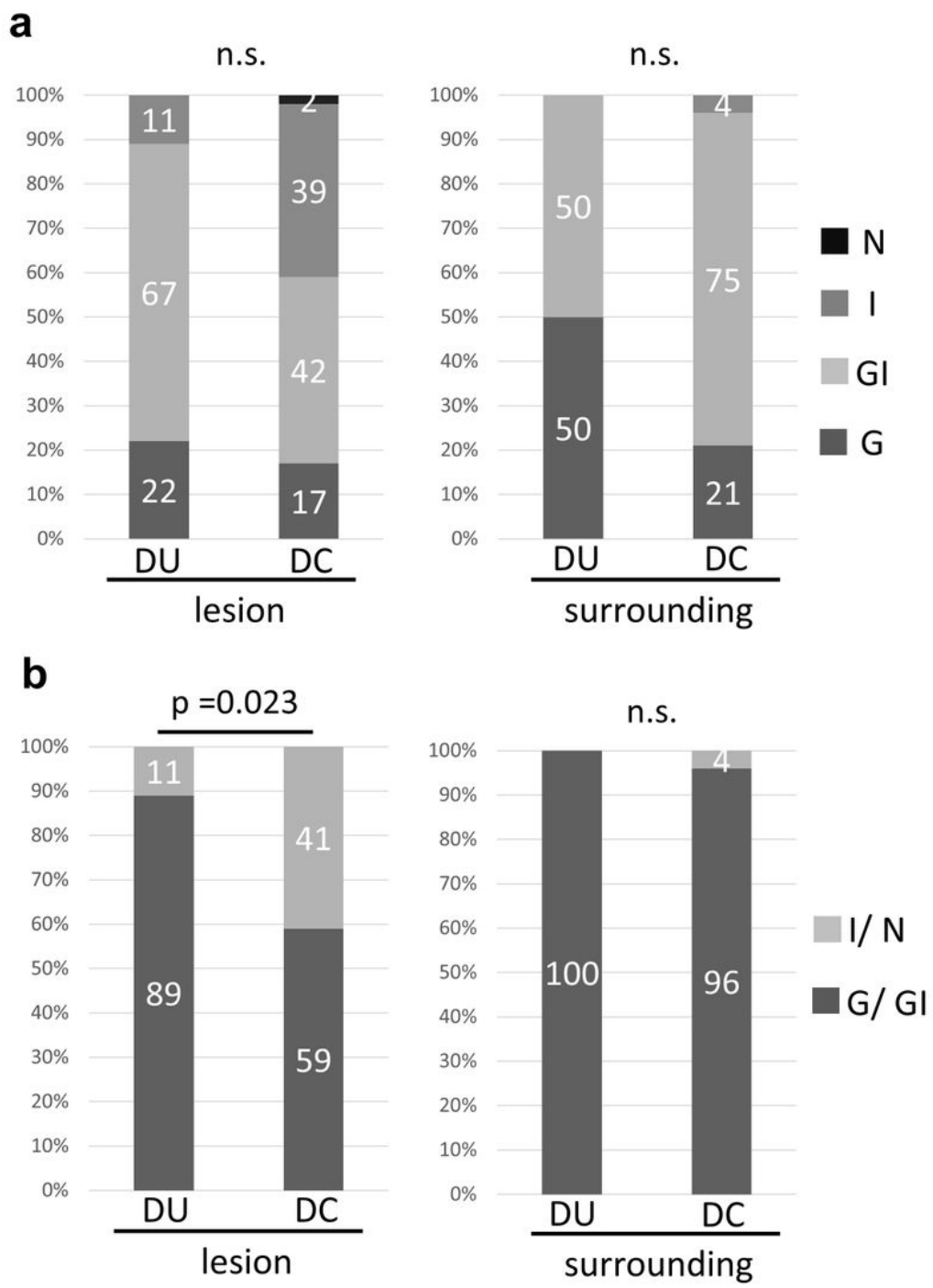


Figure 4

Difference in the mucin phenotype markers (MUC5AC, MUC6, MUC2, CD10) between the DU and DC groups. a. Comparison of the mucin phenotype marker in the lesion and the surrounding mucosa between the DU and DC groups. b. Comparison of gastric type in the lesion and the surrounding mucosa between the DU and DC groups. G, gastric type; GI, gastrointestinal type; I, intestinal type; N, null type; DU, demarcation unclear; DC, demarcation clear

Figure 5

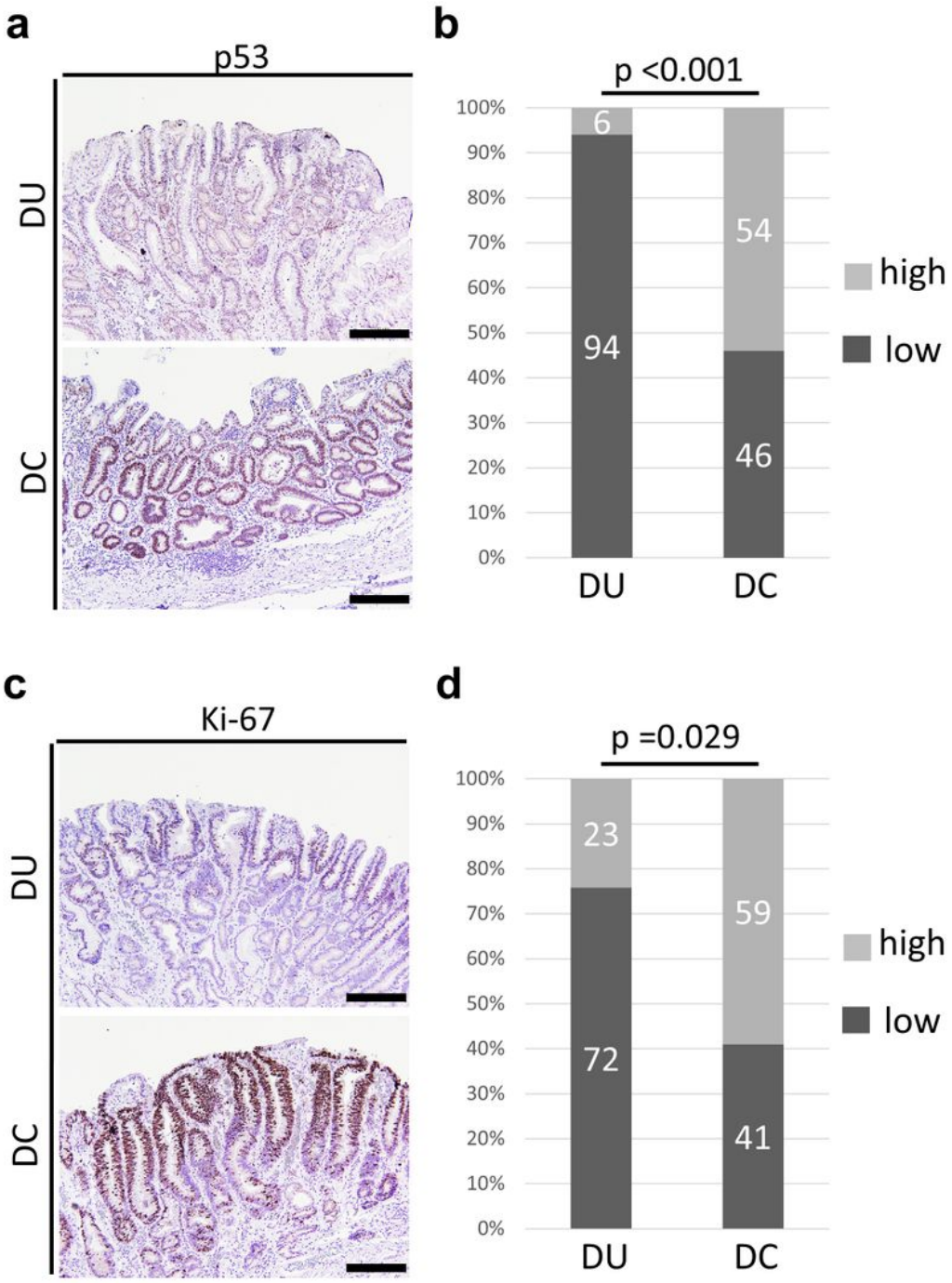


Figure 5

Differences in p53 score and Ki-67 labeling index between the DU and DC groups. a. Immunohistochemical image showing p53 positivity. b. Comparison of p53 positivity between the DU and DC groups. c. Immunohistochemical image showing Ki-67 labeling index. d. Comparison of Ki-67 labeling index between the DU and DC groups. DU, demarcation unclear; DC, demarcation clear