

## Original Article



# Clinical Implications of Histologic Heterogeneity in Endoscopic Submucosal Dissection Specimens for Differentiated-Type Early Gastric Cancer

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## ABSTRACT

**Purpose:** The clinical implications of histologic heterogeneity, defined as differentiated-type early gastric cancer (EGC) containing undifferentiated components, in endoscopic submucosal dissection (ESD) specimens remain unclear. We investigated the association of histologic heterogeneity and biopsy-detected poorly differentiated components with lymph node metastasis in differentiated-type EGC.

**Materials and Methods:** This retrospective study included 858 patients who underwent gastrectomy after ESD for differentiated-type EGC. Histologic heterogeneity in ESD specimens and its association with clinicopathological features, including lymph node metastasis, were analyzed. In addition, 88 pre-treatment biopsy specimens were reviewed for the presence of poorly differentiated components.

**Results:** Histologic heterogeneity was identified in 252 (29.4%) patients, and lymph node metastasis was detected in 46 (5.4%) patients. Histologic heterogeneity was significantly associated with higher frequencies of lymphatic invasion (73.4% vs. 50.8%,  $P<0.001$ ) and lymph node metastasis (8.3% vs. 4.1%,  $P=0.013$ ). Among patients with available biopsy specimens, poorly differentiated components identified on biopsy were observed only in lesions with histologic heterogeneity in ESD specimens and were associated with a higher frequency of lymph node metastasis (37.5% vs. 2.5%,  $P=0.005$ ).

**Conclusions:** Histologic heterogeneity in ESD specimens was associated with adverse pathological features, including lymphatic invasion and lymph node metastasis. These findings support the detailed documentation of histologic heterogeneity in ESD pathology reports and minor poorly differentiated components in biopsy specimens to assist in risk stratification and individualized treatment planning for differentiated-type EGC.

**Keywords:** Stomach neoplasms; Endoscopic submucosal dissection; Biopsy

Soomin Ahn <https://orcid.org/0000-0002-1979-4010>**Conflict of Interest**

No potential conflict of interest relevant to this article was reported.

**Author Contributions**

Conceptualization: K.K.M., A.S.; Data curation: Y.H.J., A.S.; Formal analysis: Y.H.J., A.S.; Investigation: Y.H.J., A.S.; Methodology: K.K.M., A.S.; Resources: K.T.S., L.H., M.B.H., M.Y.W., L.J.H.; Writing - original draft: Y.H.J., A.S.; Writing - review & editing: Y.H.J., K.T.S., L.H., M.B.H., M.Y.W., L.J.H., K.K.M., A.S.

**INTRODUCTION**

Gastric cancer is the fifth most common malignancy worldwide, with approximately one million new cases diagnosed annually [1]. Endoscopic submucosal dissection (ESD) is the standard treatment for early gastric cancer (EGC), with a low risk of lymph node metastasis [2-7]. Recent advances in diagnostic endoscopy, particularly image-enhanced endoscopy, have substantially improved the detection and risk stratification of early gastric neoplasms, leading to expanded indications for ESD and improved procedural outcomes. Accordingly, accurate histopathological evaluation of ESD specimens has become increasingly important for determining curative resection and the need for additional treatment [8]. Patients with risk factors for lymph node metastasis or non-curative resection are generally recommended to undergo gastrectomy with lymph node dissection [2,4,5]. Established histological risk factors include lymphovascular invasion (LVI), depth of invasion, tumor histology, and resection margin status [9].

According to current guidelines, ESD is indicated for well- or moderately differentiated EGCs meeting specific endoscopic criteria, as well as for selected undifferentiated EGCs that satisfy the strict requirements for curative resection [2]. However, histopathological examination of ESD specimens occasionally reveals the presence of undifferentiated components within predominantly differentiated tumors [10]. This phenomenon, referred to as histologic heterogeneity, describes differentiated-dominant tumors containing minor undifferentiated components [10]. Previous studies based on surgical specimens have reported that histologic heterogeneity is associated with an increased risk of lymph node metastasis compared with pure differentiated-type tumors [11-14].

However, the clinical significance of histologic heterogeneity in ESD specimens remains unclear. Because treatment decisions are primarily based on the ESD pathology, understanding the implications of histologic heterogeneity at this stage is clinically important. Therefore, the present study aimed to evaluate the clinical significance of histologic heterogeneity identified in ESD specimens in relation to lymph node metastasis in patients with differentiated-type EGC who subsequently underwent gastrectomy with lymph node dissection. In addition, we investigated whether poorly differentiated components identified in pretreatment biopsy specimens could serve as surrogate markers for histologic heterogeneity and lymph node metastasis.

**MATERIALS AND METHODS****Study population and design**

This retrospective study included patients who underwent gastrectomy following ESD for EGC at the Samsung Medical Center between May 2005 and March 2021 (n=1,207 initially identified). Patients with predominantly undifferentiated histology, indeterminate histology, or special histological types in ESD specimens were excluded. Special histological types included carcinoma with lymphoid stroma, mucinous carcinoma, and hepatoid carcinoma. Patients who underwent ESD at other hospitals or patients in which the histologic subtype and heterogeneity were not reported in the pathology report were also excluded. The final study cohort comprised 858 patients (**Fig. 1**).

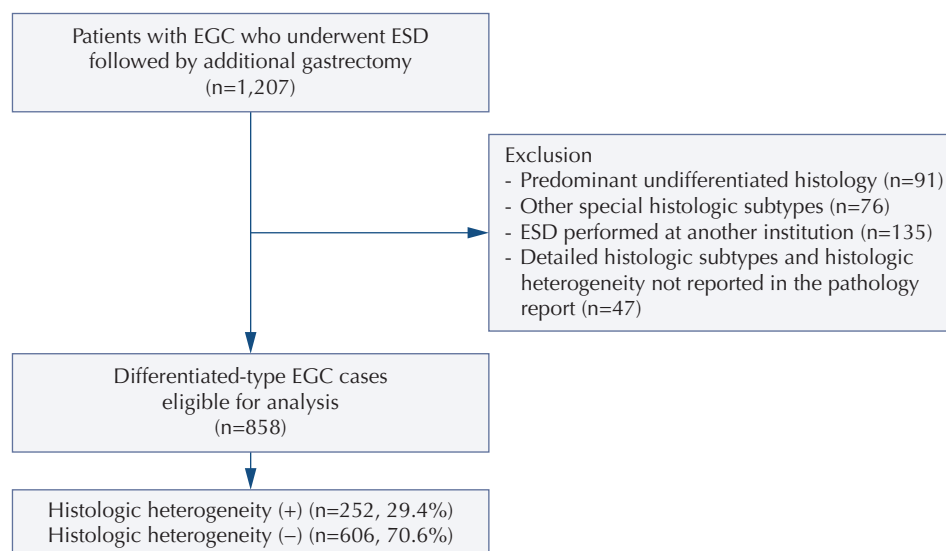


**Fig. 1.** Flow chart of patient selection and study cohort.  
EGC = early gastric cancer; ESD = endoscopic submucosal dissection.

The primary objective of this study was to evaluate the association between histologic heterogeneity in ESD specimens and lymph node metastasis. In addition, as this cohort included patients who underwent gastrectomy for various clinical reasons, not limited to non-curative ESD, we performed additional analyses based on eCura risk stratification to better assess the clinical relevance of histologic heterogeneity. Furthermore, we analyzed the association of biopsy-detected poorly differentiated components with histologic heterogeneity in ESD specimens and with lymph node metastasis in surgical specimens. This study was approved by the Institutional Review Board (IRB) of Samsung Medical Center (IRB No. 2026-03-027). The requirement for informed consent was waived by the IRB because of the retrospective nature of the study.

### Pathologic evaluation

Clinicopathological data and pathological diagnoses of ESD and surgical specimens were retrieved from electronic medical records. All ESD specimens were sectioned at 2-mm intervals and evaluated according to standard guidelines [15].

Histopathological diagnoses were made by several gastrointestinal pathologists, with the majority of cases diagnosed by an experienced gastrointestinal pathologist (K.K.M., 76%). At our institution, histopathologic diagnoses of ESD specimens have been reported in a standardized format since 2007, which includes the detailed proportions of all histologic subtypes and documentation of histologic heterogeneity [10]. Histologic heterogeneity was defined as the presence of an undifferentiated-type component, comprising  $\geq 5\%$  of the total tumor area as previously described (**Fig. 2**) [10,16]. The undifferentiated-type component was defined as poorly differentiated tubular adenocarcinoma and poorly cohesive carcinoma, including both signet and non-signet ring cell types. All specific histologic subtypes were explicitly documented. D2-40 immunohistochemistry was performed in selected cases to evaluate lymphatic invasion. Piecemeal resection is not included as a standard item in the pathology report format; therefore, in the present study, it was identified based on comments in the pathology reports when explicitly documented.

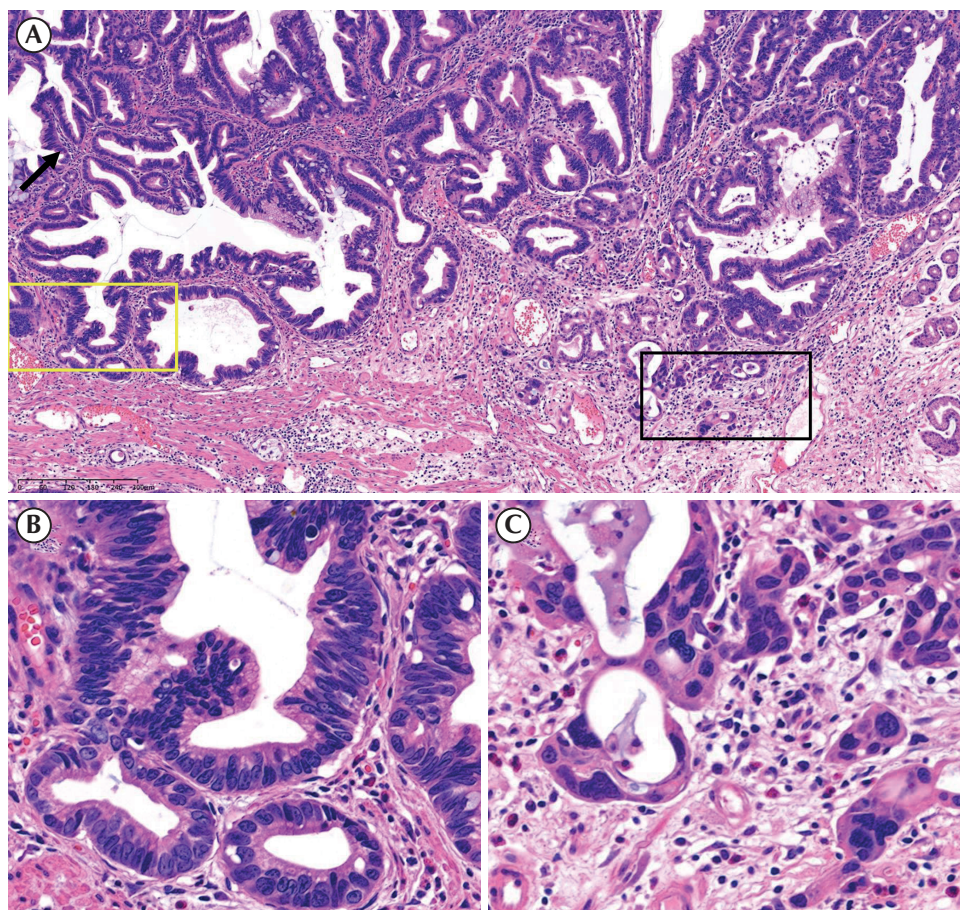


**Fig. 2.** Representative case of differentiated-type EGC with histologic heterogeneity. (A) Low-power view of the specimen (H&E, ×4). (B) High-power view of the differentiated component corresponding to the yellow box (H&E, ×20). (C) High-power view of the undifferentiated component corresponding to the black box (H&E, ×20).

EGC = early gastric cancer; H&E = hematoxylin and eosin staining.

### eCura classification

As this cohort included patients who underwent gastrectomy for various clinical reasons not limited to non-curative ESD, we performed additional analyses based on eCura risk stratification to better assess the clinical relevance of histologic heterogeneity. Patients were stratified according to the eCura classification system (A–C), based on pathology reports [2]. Additional subgroup analyses were performed in patients classified as having eCura C to evaluate the risk factors for lymph node metastasis.

### Review of initial biopsy

Preoperative biopsy specimens were evaluated in cases where slides were available. A total of 88 biopsy slides, obtained between July 2019 and February 2021, were reviewed. These samples were additionally assessed for the presence of poorly differentiated carcinoma components, which were categorized as having undifferentiated-type histology. All the biopsy slides were reviewed by an experienced gastrointestinal pathologist (A.S.). Histological classification was performed according to standardized criteria.

### Statistical analysis

Continuous variables were presented as medians with interquartile ranges (IQRs), and categorical variables as percentages. Between-group comparisons of continuous variables were performed using the Mann–Whitney U test, and comparisons for categorical variables were performed using the  $\chi^2$  test or Fisher's exact test. Additional analyses were performed to evaluate the association between the proportion of undifferentiated components and lymph node metastasis using various categorical groupings. Univariate and multivariate logistic regression analyses were performed to identify independent risk factors. A P-value <0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS statistics version 29.0.2.0 (IBM Corp., Armonk, NY, USA).

## RESULTS

### Patient characteristics

In total, 858 patients were included in the final analysis. Baseline clinical and pathological characteristics are summarized in **Table 1**. The median age was 64 years (IQR, 57–70 years), and 674 (78.6%) patients were male. Tumors were located in the antrum in 420 (49.0%), the body in 338 (39.4%), and the fundus in 100 (11.7%) patients. Multiple lesions were identified in 45 (5.2%) patients. In ESD specimens, tumor invasion was confined to the mucosa in 211 (24.6%) patients and extended into the submucosa in 647 (75.4%) patients. The depth of invasion was distributed as follows: lamina propria (n=63), muscularis mucosa (n=148), submucosa <500  $\mu$ m (n=159), and submucosa  $\geq$ 500  $\mu$ m (n=488). The median tumor size was 20 mm (IQR, 14–28 mm). Regarding histologic differentiation, 142 (16.6%) tumors were well-differentiated adenocarcinomas and 716 (83.4%) were moderately differentiated. Lymphatic invasion was identified in 493 (57.5%), venous invasion in 56 (6.5%), and perineural invasion in 7 (0.8%) patients. Resection margin involvement was observed in 235 (27.4%) patients. Histological heterogeneity was observed in 252 (29.4%) patients. Piecemeal resection was documented in the pathology reports in seven (0.8%) cases. In gastrectomy specimens, lymph node metastasis was detected in 46 (5.4%) patients.

### Association between histologic heterogeneity and clinicopathologic factors

Histologic heterogeneity was significantly associated with sex (P=0.018), tumor location (P=0.019), tumor size (P=0.007), multiplicity (P=0.015), histological differentiation (well vs. moderate differentiation, P<0.001), and lymphatic invasion (P<0.001). Tumors with histologic heterogeneity showed a higher frequency of lymphatic invasion compared to that of those without heterogeneity (73.4% vs. 50.8%). In gastrectomy specimens, histologic heterogeneity was significantly associated with lymph node metastasis (8.3% vs. 4.1%, P=0.013).

To evaluate the association between the proportion of the undifferentiated components and lymph node metastasis, additional analyses were performed using multiple proportion-based categories (0%, 5%–10%, 10%–20%, 20%–30%, 30%–40%, and 40%–50%) as well as broader categories (0%, 5%–25%, and 25%–50%) (**Supplementary Tables 1 and 2**). No clear stepwise increase in the risk of lymph node metastasis or distinct cutoff value was identified (**Supplementary Tables 1–3**).

**Table 1.** Clinicopathologic characteristics of the study population based on histologic heterogeneity

| Variable                   | No histologic heterogeneity<br>(n=606) | Histologic heterogeneity<br>(n=252) | P-value |
|----------------------------|----------------------------------------|-------------------------------------|---------|
| Age (yr)                   |                                        |                                     | 0.14    |
| >65                        | 330 (54.5%)                            | 151 (59.9%)                         |         |
| ≤65                        | 276 (45.5%)                            | 101 (40.1%)                         |         |
| Sex                        |                                        |                                     | 0.02    |
| Male                       | 489 (80.7%)                            | 185 (73.4%)                         |         |
| Female                     | 117 (19.3%)                            | 67 (26.6%)                          |         |
| Tumor location             |                                        |                                     | 0.02    |
| Fundus                     | 76 (12.5%)                             | 24 (9.5%)                           |         |
| Body                       | 252 (41.6%)                            | 86 (34.1%)                          |         |
| Antrum                     | 278 (45.9%)                            | 142 (56.3%)                         |         |
| Tumor size (mm)            |                                        |                                     | 0.007   |
| Median (quartile)          | 20.0 (14.0, 28.0)                      | 22.0 (16.0, 30.0)                   |         |
| Multiple lesions           |                                        |                                     | 0.02    |
| Absent                     | 567 (93.6%)                            | 246 (97.6%)                         |         |
| Present                    | 39 (6.4%)                              | 6 (2.4%)                            |         |
| Piecemeal resection        |                                        |                                     | 0.43    |
| Absent                     | 602 (99.3%)                            | 249 (98.8%)                         |         |
| Present                    | 4 (0.7%)                               | 3 (1.2%)                            |         |
| Depth of invasion          |                                        |                                     | 0.72    |
| Mucosa                     | 147 (24.3%)                            | 64 (25.4%)                          |         |
| Submucosa                  | 459 (75.7%)                            | 188 (74.6%)                         |         |
| Histologic differentiation |                                        |                                     | <0.001  |
| Well-differentiated        | 124 (20.5%)                            | 18 (7.1%)                           |         |
| Moderately differentiated  | 482 (79.5%)                            | 234 (92.9%)                         |         |
| Lymphatic invasion         |                                        |                                     | <0.001  |
| Absent                     | 298 (49.2%)                            | 67 (26.6%)                          |         |
| Present                    | 308 (50.8%)                            | 185 (73.4%)                         |         |
| Venous invasion            |                                        |                                     | 0.46    |
| Absent                     | 564 (93.1%)                            | 238 (94.4%)                         |         |
| Present                    | 42 (6.9%)                              | 14 (5.6%)                           |         |
| Perineural invasion        |                                        |                                     | 0.20    |
| Absent                     | 603 (99.5%)                            | 248 (98.4%)                         |         |
| Present                    | 3 (0.5%)                               | 4 (1.6%)                            |         |
| Deep margin involvement    |                                        |                                     | 0.77    |
| Absent                     | 517 (85.3%)                            | 213 (84.5%)                         |         |
| Present                    | 89 (14.7%)                             | 39 (15.5%)                          |         |
| Lateral margin involvement |                                        |                                     | 0.63    |
| Absent                     | 520 (85.8%)                            | 213 (84.5%)                         |         |
| Present                    | 86 (14.2%)                             | 39 (15.5%)                          |         |
| Microscopic ulcer          |                                        |                                     | 0.57    |
| Absent                     | 566 (93.4%)                            | 238 (94.4%)                         |         |
| Present                    | 40 (6.6%)                              | 14 (5.6%)                           |         |
| Lymph node metastasis      |                                        |                                     | 0.01    |
| Absent                     | 581 (95.9%)                            | 231 (91.7%)                         |         |
| Present                    | 25 (4.1%)                              | 21 (8.3%)                           |         |

Values are presented as number (%) or median (interquartile range).

Continuous variables were analyzed using the Mann-Whitney U test. Categorical variables were analyzed using the  $\chi^2$  test or Fisher's exact test, as appropriate.

### Histologic heterogeneity and lymph node metastasis according to the ESD curability status

Among the 858 patients, 44 were classified as eCura A (curative resection within absolute indication criteria) and 31 as eCura B (curative resection within expanded indication criteria), and one patient in each group had lymph node metastasis. In the eCura A group (n=44), five patients had histologic heterogeneity, and one of these patients (with intramucosal tumor with a size of 4 cm and histologic heterogeneity, without ulceration) developed lymph node metastasis. In the eCura B group (n=31), four patients had histologic heterogeneity. However, lymph node metastasis occurred in one patient without histologic heterogeneity. No statistically significant association was observed between histological heterogeneity and lymph node metastasis in the eCura A and B groups. The remaining 783 patients were classified as eCura C (non-curative resection), and histologic heterogeneity was significantly associated with lymph node metastasis in this group (odds ratio, 1.93; 95% confidence interval, 1.04–3.56;  $P=0.036$ ).

### Risk factors for lymph node metastasis

In the univariate analysis, lymph node metastasis was significantly associated with piecemeal resection ( $P=0.019$ ), depth of invasion ( $P=0.033$ ), histologic differentiation ( $P=0.037$ ), histologic heterogeneity ( $P=0.015$ ), lymphatic invasion ( $P<0.001$ ), venous invasion ( $P=0.018$ ), and deep margin involvement ( $P=0.011$ ) (**Table 2**). In the multivariate analysis, lymphatic invasion ( $P=0.006$ ) and piecemeal resection ( $P=0.007$ ) were independently associated with lymph node metastasis. Other clinicopathological variables, including histologic heterogeneity, were not significant in the multivariate model (**Table 3**).

We conducted additional univariate and multivariate analyses, specifically for the eCura C group. Consistent with the overall results, only lymphatic invasion and piecemeal resection were statistically significant in multivariate analysis (**Supplementary Tables 4 and 5**).

Furthermore, to evaluate the potential overlap between histologic differentiation and heterogeneity, separate multivariate analyses including each variable individually were performed. The overall findings remained unchanged, with lymphatic invasion and piecemeal resection remaining independent predictors of lymph node metastasis (**Supplementary Tables 6 and 7**).

### Biopsy-based analysis of poorly differentiated components

Pre-treatment biopsy slides were available for 88 patients. Among these, eight patients (9.1%) showed poorly differentiated components in the biopsy specimens (**Fig. 3**). The proportion of poorly differentiated components ranged from 5% to 20% in the biopsy specimens. All eight cases showed histologic heterogeneity in the corresponding ESD specimens, with undifferentiated components ranging from 5% to 30%. Patients with poorly differentiated components in biopsy specimens showed significantly higher undifferentiated proportion in ESD specimens than that of those without poorly differentiated components in biopsy specimens (median 10% vs. 0%,  $P<0.001$ ). Lymph node metastasis occurred more frequently in patients with poorly differentiated components in biopsy specimens than in those without poorly differentiated components (37.5% vs. 2.5%,  $P=0.005$ ), indicating an association between biopsy-detected poorly differentiated components and lymph node metastasis (**Table 4**).

**Table 2.** Univariate analysis of risks for lymph node metastasis

| Variable                   | Lymph node status     |                    | Univariate OR (95% CI) | P-value |
|----------------------------|-----------------------|--------------------|------------------------|---------|
|                            | No metastasis (n=812) | Metastasis (n=46)  |                        |         |
| Age (yr)                   |                       |                    |                        | 0.08    |
| >65                        | 461 (56.8%)           | 20 (43.5%)         | 1                      |         |
| ≤65                        | 351 (43.2%)           | 26 (56.5%)         | 1.707 (0.938–3.109)    |         |
| Sex                        |                       |                    |                        | 0.06    |
| Male                       | 643 (79.2%)           | 31 (67.4%)         | 1                      |         |
| Female                     | 169 (20.8%)           | 15 (32.6%)         | 1.841 (0.971–3.489)    |         |
| Tumor location             |                       |                    |                        | 0.40    |
| Fundus                     | 96 (11.8%)            | 4 (8.7%)           | 1                      |         |
| Body                       | 323 (39.8%)           | 15 (32.6%)         | 1.115 (0.361–3.437)    |         |
| Antrum                     | 393 (48.4%)           | 27 (58.7%)         | 1.649 (0.564–4.824)    |         |
| Tumor size (mm)            |                       |                    |                        | 0.08    |
| Median (quartile)          | 20.0 (14.0, 28.0)     | 23.0 (18.0, 32.75) | 1.021 (0.998–1.044)    |         |
| Multiple lesions           |                       |                    |                        | NA*     |
| Absent                     | 767 (94.5%)           | 46 (100%)          | 1                      |         |
| Present                    | 45 (5.5%)             | 0 (0%)             | -                      |         |
| Piecemeal resection        |                       |                    |                        | 0.02    |
| Absent                     | 807 (99.4%)           | 44 (95.7%)         | 1                      |         |
| Present                    | 5 (0.6%)              | 2 (4.3%)           | 7.336 (1.384–38.881)   |         |
| Depth of invasion          |                       |                    |                        | 0.03    |
| Mucosa                     | 206 (25.4%)           | 5 (10.9%)          | 1                      |         |
| Submucosa                  | 606 (74.6%)           | 41 (89.1%)         | 2.787 (1.087–7.149)    |         |
| Histologic differentiation |                       |                    |                        | 0.04    |
| Well-differentiated        | 140 (17.2%)           | 2 (4.3%)           | 1                      |         |
| Moderately differentiated  | 672 (82.8%)           | 44 (95.7%)         | 4.583 (1.098–19.128)   |         |
| Histologic heterogeneity   |                       |                    |                        | 0.02    |
| Absent                     | 581 (71.6%)           | 25 (54.3%)         | 1                      |         |
| Present                    | 231 (28.4%)           | 21 (45.7%)         | 2.113 (1.160–3.849)    |         |
| Lymphatic invasion         |                       |                    |                        | <0.001  |
| Absent                     | 358 (44.1%)           | 7 (15.2%)          | 1                      |         |
| Present                    | 454 (55.9%)           | 39 (84.8%)         | 4.393 (1.942–9.939)    |         |
| Venous invasion            |                       |                    |                        | 0.02    |
| Absent                     | 763 (94.0%)           | 39 (84.8%)         | 1                      |         |
| Present                    | 49 (6.0%)             | 7 (15.2%)          | 2.795 (1.189–6.571)    |         |
| Perineural invasion        |                       |                    |                        | 0.32    |
| Absent                     | 806 (99.3%)           | 45 (97.8%)         | 1                      |         |
| Present                    | 6 (0.7%)              | 1 (2.2%)           | 2.985 (0.352–25.326)   |         |
| Deep margin involvement    |                       |                    |                        | 0.01    |
| Absent                     | 697 (85.8%)           | 33 (71.7%)         | 1                      |         |
| Present                    | 115 (14.2%)           | 13 (28.3%)         | 2.388 (1.220–4.673)    |         |
| Lateral margin involvement |                       |                    |                        | 0.25    |
| Absent                     | 691 (85.1%)           | 42 (91.3%)         | 1                      |         |
| Present                    | 121 (14.9%)           | 4 (8.7%)           | 0.544 (0.192–1.544)    |         |
| Microscopic ulcer          |                       |                    |                        | 0.26    |
| Absent                     | 759 (93.5%)           | 45 (97.8%)         | 1                      |         |
| Present                    | 53 (6.5%)             | 1 (2.2%)           | 0.318 (0.043–2.354)    |         |

OR = odds ratio; CI = confidence interval; NA = not applicable.

\*Not estimable owing to complete separation.

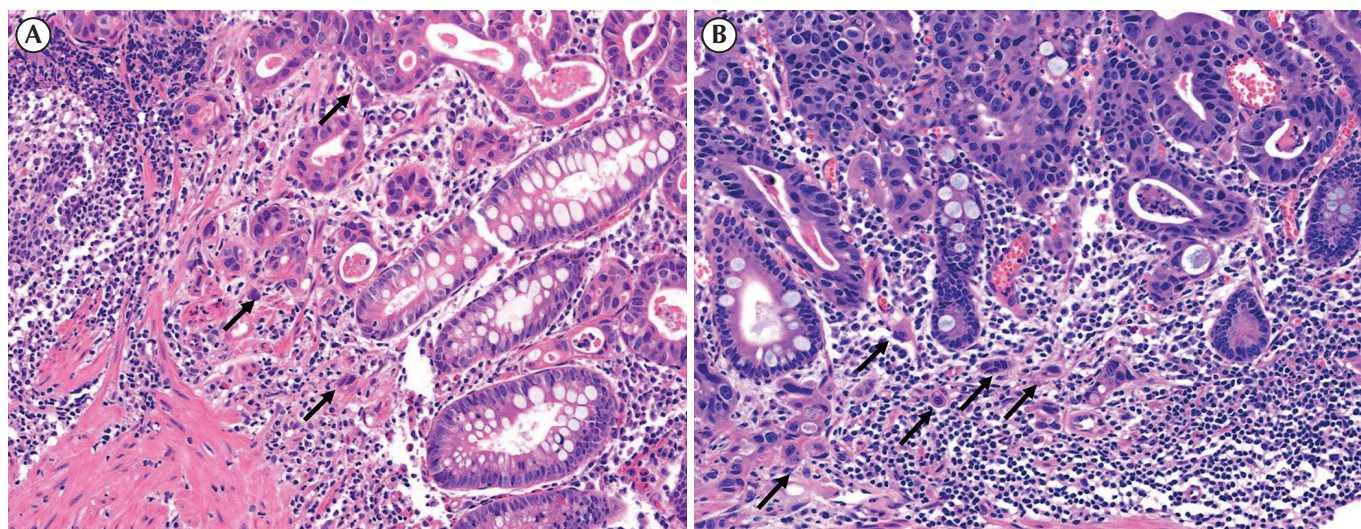


**Fig. 3.** Poorly differentiated component in a biopsy specimen and corresponding histologic heterogeneity in the ESD specimen (H&E,  $\times 10$ ). (A) Biopsy specimen with scattered single tumor cells representing a poorly differentiated component (black arrows). (B) Corresponding ESD specimen showing histologic heterogeneity with dispersed single tumor cells and small poorly differentiated tumor clusters (black arrows). ESD = endoscopic submucosal dissection; H&E = hematoxylin and eosin staining.

**Table 3.** Multivariate analysis of risk factors for lymph node metastasis

| Factor                                   | OR (95% CI)           | P-value |
|------------------------------------------|-----------------------|---------|
| Depth of invasion (mucosa vs. submucosa) | 1.927 (0.722–5.142)   | 0.19    |
| Histologic differentiation               | 2.792 (0.644–12.100)  | 0.17    |
| Histologic heterogeneity                 | 1.646 (0.881–3.075)   | 0.12    |
| Lymphatic invasion                       | 3.376 (1.413–8.062)   | 0.006   |
| Venous invasion                          | 2.008 (0.822–4.905)   | 0.13    |
| Deep margin involvement                  | 1.572 (0.771–3.204)   | 0.21    |
| Piecemeal resection                      | 12.790 (2.001–81.736) | 0.007   |

OR = odds ratio; CI = confidence interval.

**Table 4.** Association between poorly differentiated component in available biopsy slides and lymph node metastasis in surgical specimens

| Lymph node status | Poorly differentiated component in the biopsy |           | P-value |
|-------------------|-----------------------------------------------|-----------|---------|
|                   | Absent                                        | Present   |         |
| No metastasis     | 78 (97.5%)                                    | 5 (62.5%) | 0.005   |
| Metastasis        | 2 (2.5%)                                      | 3 (37.5%) |         |

## DISCUSSION

ESD is the standard treatment for differentiated-type EGC with a low risk of lymph node metastasis. However, some lesions initially diagnosed as differentiated-type gastric cancer on endoscopic biopsy were subsequently found to contain mixed undifferentiated components in ESD specimens, a phenomenon that has long been described as histologic heterogeneity [10,16]. Previous studies based on surgically resected specimens reported that histologic heterogeneity is significantly associated with lymph node metastasis in differentiated-predominant EGCs, including both intramucosal and submucosally invasive tumors [12,16]. However, data on the clinical significance of histologic heterogeneity in ESD specimens are limited. Previously, our institution evaluated the clinical significance

of histologic heterogeneity in differentiated-predominant EGCs treated with ESD, most of which met the curative resection criteria [17]. Histologic heterogeneity is associated with adverse pathologic features, including lymphatic and submucosal invasion [17]. However, the long-term outcome of differentiated-predominant EGCs with histologic heterogeneity is favorable compared to that of pure differentiated EGCs, provided that the curative resection criteria are met [11,17]. In the present study, we further evaluated the clinical significance of histologic heterogeneity, including its association with lymph node metastasis, in patients with differentiated-predominant EGCs who underwent gastrectomy after ESD, most of whom met the non-curative resection criteria.

In this study, histologic heterogeneity in ESD specimens was significantly associated with lymph node metastasis and adverse clinicopathological features such as lymphatic invasion or tumor size. Although histologic heterogeneity was not an independent predictor in multivariate analysis, it was strongly associated with established adverse pathologic features, particularly lymphatic invasion, suggesting that histologic heterogeneity may reflect the underlying aggressive tumor biology rather than act as a fully independent predictor. While histologic heterogeneity was not an independent predictor of lymph node metastasis, it may provide additional information in patients with pathological findings close to the criteria for curative resection, such as submucosal invasion close to the 500- $\mu$ m cutoff or a negative but close deep-resection margin. While the Japanese gastric cancer treatment guidelines generally state that EGCs consisting of both differentiated and undifferentiated components are classified according to the quantitatively predominant histologic type, they specifically recommend classifying a lesion as eCura C if an undifferentiated component is found within the submucosally invasive part of an eCura B lesion [2]. This necessity for detailed reporting is similarly emphasized in the Korean guidelines for standardized pathology reports, which recommend a comprehensive description of all observed histological components [15]. Collectively, these results emphasize the necessity of reporting focal undifferentiated portions in standardized ESD pathology reports. Recently, tumor budding has emerged as an important histopathologic prognostic marker in gastric cancer, particularly in predicting the risk of lymph node metastasis in EGC [18,19]. In colorectal cancer, tumor budding is defined as isolated single tumor cells or small clusters of up to four tumor cells at the invasive front [20]. After incorporating histologic heterogeneity into our institutional pathology reporting system, particular attention has been paid to the presence of dispersed single tumor cells or small clusters of tumor cells corresponding to tumor budding; such findings have been reported as histologic heterogeneity. Therefore, lesions showing tumor budding may morphologically overlap with cases classified as histologically heterogeneous in our reporting system, suggesting a potential overlap between these two concepts. However, histologic heterogeneity is a broader concept that encompasses lesions with undifferentiated histologic components, including solid-type poorly differentiated adenocarcinoma, according to the World Health Organization classification [21], indicating that the two entities are not completely interchangeable. These observations suggest that further studies are needed to establish more precise definitions and standardized terminologies for histologic heterogeneity and tumor budding in EGC.

This study also reviewed pretreatment biopsy specimens as a surrogate marker for histologic heterogeneity in ESD specimens and the associated lymph node metastasis risk. The presence of a poorly differentiated component in the biopsy specimens was associated with lymph node metastasis, and all biopsy-positive cases showed histologic heterogeneity in the ESD

specimens. This suggests that even a minor poorly differentiated component identified in the biopsy material may indicate underlying tumor heterogeneity in the subsequent ESD material. In routine practice, histological classification of endoscopic biopsy specimens can occasionally be challenging because well-differentiated areas and poorly differentiated components may coexist within the same lesion, making subtype classification difficult. In such cases, the diagnosis is generally based on the dominant histological type. However, even when the dominant histology is differentiated, documenting the presence of a minor poorly differentiated component in the biopsy report may provide clinically useful information. This approach is consistent with the Korean standardized pathology reporting guidelines for gastric cancer, which recommend reporting the histological components or subtypes associated with poor prognosis, such as poorly cohesive carcinoma, poorly differentiated tubular adenocarcinoma, and micropapillary features, regardless of their proportion [15].

This study has some limitations. First, this was a retrospective study conducted at a single institution over a long period. During this period, the indications for ESD expanded and changed, resulting in a heterogeneous study population and a relatively small number of eCura A and B cases. In the present cohort, subgroup analyses of eCura A and eCura B were limited because of the small sample size. The clinical significance of histological heterogeneity in borderline cases should be further evaluated in larger cohorts. Second, the study only included patients who underwent gastrectomy after ESD rather than all patients treated with ESD, which may have resulted in a selection bias. In our cohort, a large proportion of patients had high-risk pathological features such as deep submucosal invasion and/or LVI. This cohort selection may have limited the ability to detect the independent effects of other established risk factors for lymph node metastasis, including depth of invasion, in the multivariate analysis. In addition, additional analyses according to the proportion of undifferentiated components did not identify a clear cutoff associated with an increased risk of lymph node metastasis. However, this finding may be partially attributed to the limited number of cases in each subgroup. Future studies with larger, unselected ESD cohorts are required to validate our findings. Third, the biopsy-based analysis was limited by the small number of available biopsy slides, largely because many older biopsy slides were no longer available for review after institutional storage. Furthermore, only a small number of the biopsy specimens contained poorly differentiated components. Therefore, although statistical analyses were performed, these findings should be interpreted with caution because of the limited sample size and need for validation in larger cohorts. Fourth, the analysis of ESD specimens was based primarily on the original pathology reports rather than on slide reviews. To ensure diagnostic reliability, our institution employed a standardized reporting format and all cases were evaluated by gastrointestinal pathologists, with the majority being diagnosed or reviewed in consultation with a single expert. Despite these limitations, our study highlights the clinical relevance of histologic heterogeneity in ESD specimens of differentiated-type EGC, particularly in terms of the risk of lymph node metastasis.

In conclusion, histologic heterogeneity in ESD specimens and minor poorly differentiated components in biopsy specimens were associated with lymph node metastasis and may provide clinically meaningful information for risk stratification and individualized treatment planning in differentiated-type EGC.

## SUPPLEMENTARY MATERIALS

### Supplementary Table 1

Univariate analysis of proportion of undifferentiated component and lymph node status<sup>\*,†</sup>

### Supplementary Table 2

Univariate analysis of proportion of undifferentiated component and lymph node status<sup>\*,†</sup>

### Supplementary Table 3

Analysis of proportion of undifferentiated component and lymph node status

### Supplementary Table 4

Univariate analysis of risks for lymph node metastasis on eCura C group

### Supplementary Table 5

Multivariate analysis of risk factors for lymph node metastasis on eCura C group

### Supplementary Table 6

Multivariate analysis of risk factors for lymph node metastasis without histologic differentiation

### Supplementary Table 7

Multivariate analysis of risk factors for lymph node metastasis without histologic heterogeneity

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