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# Significance of MUC1 expression in biopsy specimens of submucosal invasive gastric carcinoma: The association with lymph node metastasis

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**Abstract.** Mucin 1, cell surface associated (MUC1) is a tumor-associated glycoprotein that has been reported to have an important role in lymphatic invasion and metastasis. The present study aimed to investigate the significance of MUC1 expression in endoscopic biopsy specimens of submucosal invasive gastric carcinomas and the association with lymph node metastasis. The clinicopathological features of 144 cases of surgically resected submucosal invasive gastric carcinomas and their paired endoscopic biopsy specimens were reviewed. Immunohistochemical staining for MUC1 was performed for the 144 endoscopic biopsy specimens. Positive MUC1 expression was identified in 70 (49%) cases. In addition, univariate analysis revealed that MUC1 expression was significantly associated with the presence of poorly-differentiated (P=0.001) and poorly-cohesive (P=0.015) carcinoma cells, undifferentiated type by Japanese classification (P<0.001), diffuse type of Lauren classification (P<0.001) and lymph node metastasis (P=0.024). By multivariate analysis, diffuse type of Lauren classification (P<0.001) and lymph node metastasis (P=0.035) were identified as independent factors for MUC1 expression. Furthermore, MUC1 expression (P=0.007), tumor size (P=0.018) and lymphatic invasion (P<0.001) were demonstrated to be independent factors for lymph node metastasis under multivariate analysis. In conclusion, the results of the present study indicated that positive MUC1 expression in endoscopic biopsy specimens may be a predictive factor of lymph node metastasis in submucosal invasive gastric carcinoma.

## Introduction

Early gastric cancer (EGC) is defined as invasive gastric cancer that does not invade beyond the submucosa, irrespective of lymph node metastasis. EGC has an excellent prognosis, with a 5-year survival rate of >90% (1). There are two treatment options for EGC, endoscopic submucosal dissection (ESD) or surgical resection with lymph node dissection. Treatment methods are selected according to the probability of lymph node metastasis (2). In addition to lymph node metastasis, the size of the tumor, presence or absence of an ulcer, lymphovascular invasion and histological type are all used to determine which method of treatment should be undertaken (2).

Absolute indications for the use of ESD were defined as differentiated mucosal cancer without ulceration and tumors <2 cm in size. The expanded version of the guidelines included the following criteria: i) differentiated mucosal cancer without ulceration and tumor >2 cm in size; ii) differentiated mucosal cancer with ulceration and a tumor <3 cm in size; or iii) differentiated submucosal cancer with a tumor <3 cm in size and a submucosal invasion depth of <500 $\mu$m (3). Based on a study by Hirasawa *et al* (4), the guidelines for ESD were further expanded and it is now accepted that carcinoma of the undifferentiated type, without ulceration and with a tumor size of <2 cm in size is included in the Japanese Gastric Cancer Treatment Guidelines 2010 (ver. 3) (4,5).

ESD guidelines were previously based on hematoxylin and eosin staining; however, numerous studies have reported that the expression of cell adhesion molecules, cell surface molecules, membrane-associated mucin phenotypes and collagen phenotypes are associated with lymph node metastasis and prognosis of carcinomas in multiple organs (6-15). Mucin 1, cell surface associated (MUC1) is a transmembrane member of the mucin family and has been reported to be associated with metastatic progression (16). The present study aimed to investigate MUC1 expression using immunohistochemistry in endoscopic biopsy specimens from submucosal invasive gastric carcinomas in order to determine whether MUC1 was a potential predictor of lymph node metastasis. In addition, the present study examined the association between MUC1 expression and clinicopathological variables.

## Materials and methods

*Patient and tissue specimens*. The present study included 144 patients with submucosal invasive gastric carcinomas who underwent surgical resection with lymph node dissection following endoscopic biopsies between August 2005 and

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*Key words:* mucin 1, cell surface associated, lymph node metastasis, gastric carcinoma

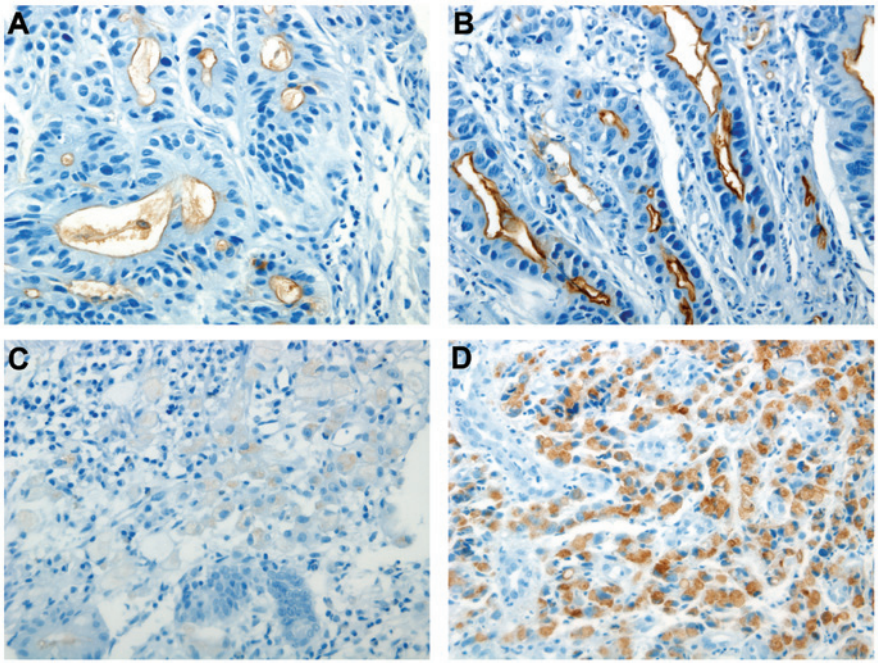


Figure 1. Immunohistochemical staining for MUC1. (A) Moderate luminal expression; (B) strong luminal expression; (C) weak cytoplasmic expression; and (D) moderate cytoplasmic expression of MUC1. Immunohistochemical results of (A) and (C) are negative and (B) and (D) are positive. MUC1, mucin 1, cell surface associated.

December 2012 at Konkuk University Medical Center (Seoul, Korea). All cases were reviewed according to the current guidelines from the World Health Organization 2010 classifications (17). Immunohistochemical staining for MUC1 was performed in endoscopic biopsy specimens and the association of MUC1 immunoreactivity with the following clinicopathological characteristics of patients was investigated: Tumor size, histological type, gross type, depth of invasion, lymphovascular invasion, perineural invasion, Lauren classification, mucin phenotype, p53 immunoreactivity, MUC1 immunoreactivity and lymph node metastasis in surgically resected submucosal invasive gastric carcinomas. The present study was approved by the Institutional Review Board of Konkuk University Medical Center.

*Immunohistochemistry*. The formalin-fixed, paraffin-embedded tissue blocks were sectioned at 3-$\mu$m thickness and immunohistochemical staining was performed using an iVIEW DAB detection kit and a BenchMark XT staining instrument (Ventana Medical System, Inc., Tucson, AR, USA). Immunohistochemical staining was performed using rabbit polyclonal MUC1 (dilution, 1:400; catalog no., RB9222P0) primary antibodies in endoscopic biopsy specimens; and mouse monoclonal CD10 (clone, 56C6; dilution, 1:50; catalog no., MS728S0), MUC2 (clone, 996/1; dilution, 1:2,000; catalog no., MS1729P0), MUC5AC (clone, 45M1; dilution, 1:2,000; catalog no., MS145P0), MUC6 (clone, CLH5; dilution, 1:200; catalog no., MS1153S0) and p53 (clone, DO-7; dilution, 1:500; catalog no., MS186P0) primary antibodies in surgically resected specimens. All primary antibodies were purchased from Thermo Fisher Scientific (Fremont, CA, USA). Mucin phenotypes were evaluated through CD10, MUC2, MUC5AC and MUC6 immunohistochemical staining in order to classify each as gastric type, intestinal type, mixed type or unclassified type. Positive reactivity for MUC1 was defined as strong luminal immunoreactivity or more than moderate cytoplasmic immunoreactivity in >5% of the tumor cells (Fig. 1). In addition, positive reactivity for CD10, MUC2, MUC5AC and MUC6 was defined as positive immunoreactivity in >10% of tumor cells and for p53, in >5% of tumor cells.

*Statistical analysis*. MUC1 immunoreactivity in association with clinicopathological parameters was examined using the $\chi^2$ and Student's *t* tests. The lymph node metastasis in association with MUC1 immunoreactivity and clinicopathological parameters was examined using the $\chi^2$ test and Student's *t* test. Multivariate analysis was performed using logistic regression. $P<0.05$ was considered to indicate a statistically significant difference between values. Values are presented as the mean ± standard deviation. All statistical analyses were performed using SPSS version 20.0 (International Business Machines, Armonk, NY, USA).

## Results

*Clinicopathological variables*. There were 17, 71, 24, 14 and 18 cases of well-, moderately- and poorly-differentiated tubular adenocarcinomas, mixed adenocarcinomas and poorly-cohesive carcinomas with biopsy specimens, respectively. In total, there were 89, 40, 9, 5 and 1 cases of tubular adenocarcinomas, mixed adenocarcinomas, poorly-cohesive carcinomas, medullary carcinomas and mucinous carcinoma with surgically resected submucosal invasive cancers, respectively. Of the total 144 submucosal invasive gastric carcinomas, 6 cases were surgically resected following ESD due to a submucosal invasion depth ≥500 $\mu$m or involvement of the ESD resection margin. The mean age of the patients was 61.7 ± 11.5 years and the study group was comprised of 97 men and 47 women. The

Table I. Univariable analysis of lymph node metastasis and clinicopathological features in submucosal invasive gastric carcinoma.

| Clinicopathological feature | Lymph node metastasis | | P-value |
|---|---|---|---|
| | Present (n=24) | Absent (n=120) | |
| Mean age (SD) | 60 (14.0) | 62 (11.0) | 0.436 |
| Gender ratio, male:female | 13:11 | 84:36 | 0.155 |
| Gross type | | | 0.267 |
| EGC I | 3 | 8 | |
| EGC IIa | 5 | 21 | |
| EGC IIb | 1 | 24 | |
| EGC IIc | 12 | 57 | |
| EGC III | 3 | 10 | |
| Endoscopic biopsy histology | | | 0.246 |
| Well-differentiated tubular adenocarcinoma | 2 | 15 | |
| Moderately-differentiated tubular adenocarcinoma | 9 | 62 | |
| Poorly-differentiated tubular adenocarcinoma | 8 | 16 | |
| Poorly-cohesive carcinoma | 3 | 15 | |
| Mixed adenocarcinoma | 2 | 12 | |
| Mean tumor size, cm (SD) | 5.0 (2.4) | 3.4 (1.8) | 0.005 |
| Mean submucosal invasion depth, $\mu$m (SD) | 1815 (997) | 1473 (1101) | 0.161 |
| Level of submucosal invasion | | | 0.018 |
| SM1 | 6 | 38 | |
| SM2 | 2 | 36 | |
| SM3 | 15 | 41 | |
| Level of submucosal invasion by Japanese classification | | | 0.6 |
| <500 $\mu$m | 4 | 27 | |
| ≥500 $\mu$m | 20 | 93 | |
| Presence of poorly differentiated cells | | | 0.038 |
| Yes | 10 | 25 | |
| No | 14 | 95 | |
| Presence of poorly cohesive cells | | | 1.0 |
| Yes | 5 | 27 | |
| No | 19 | 93 | |
| Tumor differentiation by Japanese classification | | | 0.111 |
| Differentiated | 11 | 77 | |
| Undifferentiated | 13 | 43 | |
| Endoscopic submucosal dissection indication | | | 0.356 |
| Expended indication | 0 | 9 | |
| Surgery | 24 | 111 | |
| MUC1 immunohistochemistry | | | 0.024 |
| Positive | 17 | 53 | |
| Negative | 7 | 67 | |
| p53 immunohistochemistry | | | 0.254 |
| Positive | 7 | 51 | |
| Negative | 16 | 64 | |
| Mucin phenotype | | | 0.825 |
| Gastric | 9 | 39 | |
| Intestinal | 5 | 27 | |
| Mixed | 3 | 23 | |
| Unclassified | 7 | 27 | |
| Intestinal | 10 | 75 | |

Table I. Continued.

| Clinicopathological feature | Lymph node metastasis: Present (n=24) | Lymph node metastasis: Absent (n=120) | P-value |
|---|---|---|---|
| Lauren classification | | | 0.027 |
| Diffuse | 9 | 38 | |
| Mixed | 5 | 7 | |
| Intestinal vs. diffuse | | | 0.906[a] |
| Intestinal vs. mixed | | | 0.053[a] |
| Diffuse vs. mixed | | | 0.399[a] |
| Lymphatic invasion | | | <0.001 |
| Present | 20 | 26 | |
| Not identified | 4 | 94 | |
| Vascular invasion | | | 0.59 |
| Present | 0 | 5 | |
| Not identified | 24 | 115 | |
| Perineural invasion | | | 0.522 |
| Present | 1 | 3 | |
| Not identified | 23 | 117 | |

[a]Bonferroni corrected P-value. SD, standard deviation; EGC, early gastric cancer; SM, submucosa; MUC1, mucin 1, cell surface associated.

Table II. Multivariable analysis of MUC1 expression, tumor size and lymph node metastasis in submucosal invasive gastric carcinoma.

| | Odds ratio | 95% confidence interval | P-value |
|---|---|---|---|
| MUC1 expression | 6.380 | 1.661-24.502 | 0.007 |
| Tumor size | 1.394 | 1.058-1.837 | 0.018 |
| Lymphatic invasion | 17.443 | 4.849-62.739 | <0.001 |

MUC1, mucin 1, cell surface associated.

gross types of EGCs were as follows: 11 type I, 26 type IIa, 25 type IIb, 69 type IIc and 13 type III, with a mean tumor size of 3.7±2.0 cm and a mean submucosal depth of invasion of 1,530±1,089 $\mu$m. There were 44, 38 and 56 cases of submucosal invasion level 1, 2 and 3, respectively. In 31 cases, the depth of submucosal invasion was <500 $\mu$m and in 113 cases, the depth was ≥500 $\mu$m. The level of submucosal invasion could not be measured in 6 cases due to previous ESD. The poorly-differentiated carcinoma cells were present in 35 cases and poorly-cohesive carcinoma cells were present in 32 cases of the endoscopic biopsy specimens. According to Japanese classification, undifferentiated type carcinoma includes poorly differentiated adenocarcinoma, signet ring cell carcinoma, and mucinous adenocarcinoma (5). There were 56 cases of undifferentiated type, according to the Japanese classification and among them, 9 cases were included in the expanded guidelines of ESD. There were 24 cases of lymph node metastasis. Immunohistochemical staining for MUC1 and p53 was positive in 70 and 58 cases, respectively. A total of 48, 32, 26 and 34 cases were classified into gastric, intestinal, mixed and unclassified mucin phenotypes. However, immunohistochemistry was unable to be performed for p53 expression in 6 cases and for evaluation of the mucin phenotype in 4 cases. Furthermore, 85, 47 and 12 cases were intestinal, diffuse and mixed type according to the Lauren classification, respectively. There were 46, 5 and 4 cases of lymphatic, venous and perineural invasion, respectively (Table I).

*Lymph node metastasis and clinicopathologic features.* Lymph node metastasis was identified in 24 cases and was identified to be significantly associated with tumor size (P=0.005), the level of submucosal invasion (P=0.018), the presence of poorly-differentiated carcinoma cells (P=0.038), MUC1 expression (P=0.024), Lauren classification (P=0.027) and lymphatic invasion (P<0.001), through univariate analysis (Table I). However, lymph node metastasis was not found to be associated with histologic type (P=0.246) (Table I). By contrast, following Bonferroni correction, the Lauren clas-

Table III. Univariable analysis of MUC1 expression and clinicopathological features in submucosal invasive gastric carcinoma.

| | MUC1 immunoreactivity | | |
|---|---|---|---|
| | Positive (n=70) | Negative (n=74) | P-value |
| Presence of poorly differentiated cells | | | 0.001 |
| Yes | 26 | 9 | |
| No | 44 | 65 | |
| Presence of poorly cohesive cells | | | 0.015 |
| Yes | 22 | 10 | |
| No | 48 | 64 | |
| Tumor differentiation | | | <0.001 |
| Differentiated | 31 | 57 | |
| Undifferentiated | 39 | 17 | |
| Lauren classification | | | <0.001 |
| Intestinal | 29 | 56 | |
| Diffuse | 34 | 13 | |
| Mixed | 7 | 5 | |
| Intestinal vs. diffuse | | | <0.001[a] |
| Intestinal vs. mixed | | | 0.363[a] |
| Diffuse vs. mixed | | | 1.000[a] |
| Lymph node metastasis | | | 0.024 |
| Yes | 17 | 7 | |
| No | 53 | 67 | |

[a]Bonferroni corrected P-value. MUC1, mucin 1, cell surface associated.

Table IV. Multivariable analysis of MUC1 expression and clinicopathological features in submucosal invasive gastric carcinoma.

| | Odds ratio | 95% confidence interval | P-value |
|---|---|---|---|
| Lauren classification | | | |
| Intestinal vs. diffuse | 5.158 | 2.255-11.798 | <0.001 |
| Intestinal vs. mixed | 2.028 | 0.548-7.501 | 0.289 |
| Lymph node metastasis | 3.211 | 1.088-9.473 | 0.035 |

MUC1, mucin 1, cell surface associated.

sification was not significantly associated with lymph node metastasis: Intestinal type vs. diffuse type, P=0.906; intestinal vs. mixed, P=0.053; and diffuse vs. mixed, P=0.339, according to the Bonferroni corrected P-value. The following three factors, MUC1 expression (P=0.007), size of tumor (P=0.018), and lymphatic invasion (P<0.001), were identified as independent risk factors for lymph node metastasis (Table II).

*MUC1 expression and clinicopathological features*. A total of 70 cases were positive for MUC1 immunohistochemical staining. The positive MUC1 expression was significantly associated with the presence of poorly-differentiated carcinoma cells (P=0.001), poorly-cohesive carcinoma cells (P=0.015), undifferentiated type (P<0.001), diffuse type under the Lauren classification (P<0.001) and lymph node metastasis (P=0.024) (Table III). The following two factors, diffuse type under the Lauren classification (P<0.001) and lymph node metastasis (P=0.035), were identified as independent factors for positive MUC1 expression (Table IV).

## Discussion

Mucins are high-molecular-weight epithelial glycoproteins that provide protection and lubrication to epithelial surfaces; of note, the role of mucins in cell signaling has been the focus of numerous studies (7,9,10,16). MUC1 is one of the membrane-associated type mucins that is known to contribute

to epithelial cell-to-cell interactions (7). It was demonstrated that the expression of MUC1 was primarily located at the apical surface of ductal epithelia. However, MUC1 is overexpressed in metastatic disease and becomes localized throughout the cell (16). In numerous types of tumors, MUC1 expression was reported to be correlated with aggressiveness, metastatic disease and poor prognosis (9-13,16,18). MUC1 expression was reported to accelerate tumor invasion and metastasis via the impairment of E-cadherin (10), decrease the binding of p120 catenin to E-cadherin (18), upregulate matrix metalloproteinase 13 expression (13) and activate Wnt/β-catenin abnormally (9). Furthermore, MUC1 expression was demonstrated to be associated with metastatic progression in the gastrointestinal system. However, in gastric cancer, it was reported that the expression of MUC1 was not limited to metastatic disease, but also highly expressed in the majority of isolated cancer cells invading throughout the stroma of the primary tumor (16). This therefore indicated that MUC1 may be involved in initiating the spread of cancer.

In previous studies, MUC1 expression was identified in >50% of differentiated and undifferentiated gastric carcinomas. In addition, MUC1-positive staining appeared to be associated with better tumor differentiation, as the majority of studies suggest that MUC1 expression is associated with lymphatic invasion, nodal metastasis and poor prognosis (19). In the present study, MUC1 expression was identified in 31 cases of 88 differentiated type carcinomas and in 39 cases of 56 undifferentiated type carcinomas (P<0.001). These results differed from those of previous studies, this may be due to the difference in immunohistochemical methods and analysis (12,19). In the present study, MUC1 positivity was significantly associated with the presence of poorly-differentiated carcinoma cells (P=0.001), poorly-cohesive carcinoma cells (P=0.015), the undifferentiated type according to the Japanese classification system (P<0.001), the diffuse type under the Lauren classification (P<0.001) and lymph node metastasis (P=0.024), as determined using univariate analysis. The results of the multivariate analysis demonstrated that MUC1 expression was associated with the diffuse type under the Lauren classification system (P<0.001) and lymph node metastasis (P=0.035). These results suggested that MUC1-positive staining may be associated with poorly-differentiated carcinoma cells and poorly-cohesive carcinoma cells, which invade throughout the stroma of the primary tumor, as well as lymph node metastasis.

MUC1 immunohistochemical staining patterns are divided into the luminal and cytoplasmic patterns. The present study revealed 70 positive cases for MUC1 immunohistochemical staining out of 144 total cases (48.6%). Among them, 39, 20 and 11 cases revealed cytoplasmic, luminal and mixed patterns of immunohistochemical staining, respectively. Of the 39 total undifferentiated type cases, 32 cases revealed cytoplasmic staining and 7 cases exhibited mixed staining. In addition, 20 cases out of the 31 total differentiated type cases revealed luminal staining, 7 cases showed cytoplasmic staining and 4 cases showed mixed staining. For tumor differentiation, the immunohistochemical staining pattern for MUC1 was significantly different (P<0.001) and the undifferentiated type showed MUC1 cytoplasmic staining more frequently. Yonezawa *et al* (19) reported that the MUC1 immunohistochemical staining pattern is different depending on tumor differentiation; finding that stain mainly accumulates at the apex in papillary adenocarcinoma, mucinous adenocarcinoma, well-differentiated or moderately-differentiated adenocarcinoma, while poorly-differentiated carcinoma and signet-ring cell carcinoma demonstrated primarily cytoplasmic staining. The results of the present study strongly supported this previous study, as they indicated that the immunohistochemical pattern of MUC1 varied according to tumor differentiation in gastric adenocarcinoma.

Of the 24 cases of submucosal invasive gastric carcinoma with lymph node metastasis, 17 cases demonstrated positive MUC1 expression in the endoscopic biopsy specimens. These cases included 1, 4, 8, 1 and 3 cases of well-, moderately- and poorly-differentiated adenocarcinoma, mixed adenocarcinoma and poorly-cohesive cell carcinoma, respectively. A case of well-differentiated adenocarcinoma and 3 cases out of the 4 moderately-differentiated adenocarcinomas revealed luminal MUC1 immunohistochemical patterns while the one remaining moderately-differentiated adenocarcinoma demonstrated a mixed pattern. In addition, 6 out of 8 cases of poorly-differentiated adenocarcinomas, 1 case of mixed adenocarcinoma and all 3 poorly-cohesive cell carcinomas displayed a cytoplasmic pattern, while the remaining 2 poorly differentiated adenocarcinomas exhibited a mixed pattern. Positive MUC1 expression is important, regardless of the immunohistochemical expression region.

Hirasawa *et al* (4) found there was no lymph node metastasis in 310 patients with undifferentiated type mucosal cancer with lesions ≤2 cm and without ulcers from a population of 1,442 patients with undifferentiated mucosal cancer without ulcers. The Japanese Gastric Cancer Association accepted this study, and now undifferentiated type mucosal cancer with lesions ≤2 cm without ulcers are classified as expanded ESD indications in the Japanese Gastric Cancer Treatment Guidelines (5). According to these guidelines, the most important factors for predicting lymph node metastasis are as follows: Tumor size, tumor differentiation, ulceration, depth of submucosal invasion and lymphovascular invasion. The present study included 9 cases that underwent expanded ESD and lymph node metastasis was not identified in these cases. In the present study, tumor size, level of submucosal invasion, presence of poorly-differentiated carcinoma cells, MUC1 immunoreactivity and lymphatic invasion were found to be significantly associated with lymph node metastasis, as determined using univariate analysis. The results of the multivariate analysis demonstrated that tumor size, MUC1 immunoreactivity and lymphatic invasion were significantly associated with lymph node metastasis. Of note, the level of submucosal invasion was associated with lymph node metastasis; however, the depth of submucosal invasion in accordance with the Japanese classification (500 $\mu$m of submucosal invasion depth) was not significantly associated with lymph node metastasis.

In conclusion, positive MUC1 expression in endoscopic biopsy specimens may be a predictive factor of lymph node metastasis in submucosal invasive gastric carcinoma. More large studies, including cases of expanded ESD indication, are required in order to determine whether MUC1 immunohistochemistry may be used for selecting between ESD and surgical resection.

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