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Claudin 18.2 Expression in Gastric Tumors and Other Tumor Types With Gastric Epithelium-like Differentiation

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Abstract

Background/Aim: Claudin 18.2 is an emerging biomarker for claudin 18.2-targeted therapy. We investigated claudin 18.2 expression in diverse tumor types.

Patients and Methods: We retrospectively analyzed 67 gastric tumors (61 surgically resected and six biopsy specimens) and 73 other tumor types (69 resected and four biopsy specimens), including those from the pancreas, hepatobiliary system, lung, ovary, uterine cervix, and others. Claudin 18.2 expression and positivity (≥75% of tumor cells showing moderate to strong membranous staining) were assessed using claudin 18 immunostaining (clone 43-14A).

Results: Claudin 18.2 positivity was found in 47.8% (32/67) of gastric tumor samples. Epstein-Barr virus-associated gastric cancer showed a higher frequency of positivity (6/7, 85.7%), although not statistically significantly (p=0.216). Among gastric tumors from patients with lymph node or distant metastasis (n=20), four (20.0%) exhibited discrepancies in claudin 18.2 positivity between the primary and its metastasis. In other tumor types, claudin 18.2 positivity was more frequent in those with gastric epithelium-like differentiation, including pancreatic tumors (2/9, 22.2%), hepatobiliary carcinoma (2/8, 25.0%), invasive mucinous lung adenocarcinoma (4/5, 80.0%), and mucinous ovarian tumor (5/5, 100.0%) than in those with other histology (p<0.001). Interestingly, pancreatic tumors, potential candidates for claudin 18.2-targeted therapy, often exhibited reduced or lack of claudin 18.2 expression in the invasive component.

Conclusion: Overall, claudin 18.2 positivity occurred primarily in a significant proportion of gastric tumors and other tumors with gastric epithelium-like differentiation. Evaluating claudin 18.2 expression in all such tumors can benefit patients by guiding targeted therapy. Additionally, claudin 18.2 immunostaining serves as a lineage marker for gastric origin or gastric-like differentiation.

Keywords: Gastric cancer, claudin 18.2, claudin 18.2-positive tumors, clone 43-14A, zolbetuximab.

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Introduction

Gastric cancer is a prevalent malignancy worldwide (1). Despite significant progress in precision medicine, treatment options for gastric cancer remain limited (2). Genetic heterogeneity within gastric cancer poses challenges for targeted therapy (3). Immune checkpoint inhibitors can treat advanced, non-resectable gastric cancer, but optimal responses are often limited to a subset of gastric cancer known as 'immune hot' tumors (4, 5).

Claudin 18.2, a tight-junction molecule, is exclusively expressed in the apical and lateral sides of the normal gastric epithelium membrane, maintaining cellular polarity. Its primary function is to create a selectively permeable barrier that mediates paracellular transport (6-8). During malignant transformation, cell polarity is lost, and claudin 18.2 can become exposed on the tumor cell surface. Zolbetuximab, a first-in-class monoclonal antibody targeting claudin 18.2, mediates antibody-dependent cell-mediated cytotoxicity and complement-dependent cytotoxicity. It selectively treats claudin 18.2-positive gastric cancer (9, 10). Recent major phase III clinical trials (GLOW and SPOTLIGHT) investigated the efficacy of zolbetuximab in locally advanced, unresectable, or metastatic gastric cancer. These trials demonstrated zolbetuximab significantly improved disease-free and overall survival for patients with claudin 18.2-positive gastric cancer, defined by moderate to strong membranous positivity in over 75% of tumor cells on claudin 18 immunostaining (clone 43-14A) (11, 12).

Aberrant expression of claudin 18.2 has been reported in other tumor types, including lung adenocarcinoma, pancreatic, hepatobiliary, ovarian and uterine cervix cancer (13, 14). The efficacy of zolbetuximab in these other tumor types is currently being actively investigated (15). However, detailed histological assessments of claudin 18.2-positive tumors in different organs have not been explored.

In this study, we conducted a comprehensive investigation of claudin 18.2 expression in gastric and other tumor types using immunostaining with clone 43-14A. Additionally, we explored whether claudin 18.2 expression might serve as a lineage marker for gastric-type tumors and tumors with gastric epithelium-like differentiation.

Patients and Methods

Study population. We retrospectively collected 61 consecutive cases of gastric cancer that were surgically or endoscopically resected between January 2024 and June 2024 at Kyungpook National University Chilgok Hospital. Additionally, we included six biopsy cases in which claudin 18.2 immunostaining was performed as part of the routine diagnostic workup. Based on previous research, we hypothesized that aberrant claudin 18.2 expression might be associated with other tumor types exhibiting gastric epithelium-like differentiation (14, 16). Accordingly, we collected surgically resected samples during the same period from hepatobiliary adenocarcinoma (n=8), pancreatic tumor (n=9), mucinous ovarian carcinoma (n=5), invasive mucinous lung adenocarcinoma (n=5), and uterine cervical adenocarcinoma, human papillomavirus-independent, gastric type (n=5). The same number of non-mucinous carcinomas of ovary (n=5), lung (n=5), and uterine cervix (n=5) were included as the control group. Additionally, we randomly included endometrioid carcinoma (n=8), uterine cervix squamous cell carcinoma (n=3), papillary thyroid carcinoma (n=5), colon adenocarcinoma (n=5), esophageal squamous cell carcinoma (n=2), lymphoma (n=4), and malignancy of unknown origin (n=1). Clinicopathological data, including age, sex, tumor depth, and lymph node metastasis, were obtained from the hospital's electronic medical records.

This study was approved by the Institutional Review Board of Kyungpook National University Chilgok Hospital (no: 2023-05-041). The requirement for written-informed consent from the patients was waived because of the retrospective nature of the study.

Pathological evaluation. All tumor specimens were fixed in 10% neutral-buffered formalin and embedded in paraffin blocks. Subsequently, the paraffin blocks were sectioned into 4-μm-thick slices and stained with

hematoxylin and eosin. For surgically resected specimens, MSK and ANS reviewed all available slides, selecting representative slides for claudin 18.2 immunostaining.

Immunohistochemistry and interpretation. Formalin-fixed, paraffin-embedded block slices underwent deparaffinization and rehydration using xylene and alcohol. Subsequently, these sections were incubated with antibodies against claudin 18 (mouse monoclonal, clone 43-14A, prediluted; Roche, Basel, Switzerland). A Benchmark Ultra autostainer (Ventana Medical Systems, Inc., Tucson, AZ, USA) with OptiView DAB Detection Kit (Ventana Medical Systems) were used for the staining, following the manufacturer's protocol. In cases of gastric cancer with regional or distant metastasis, claudin 18.2 immunostaining was performed on both the primary and metastatic tumors.

MSK and ANS reviewed all the stained slides. According to cut-off criteria from pivotal clinical trials (17-19), claudin 18.2 positivity was defined as tumor showing moderate-to-strong membranous reactivity in over 75% of tumor cells. Partial, basolateral, and apical membranous staining, as well as complete, circumferential membranous staining, were all considered claudin 18.2 expression. Cytoplasmic or granular staining patterns were disregarded. We also graded both the intensity (0, 1+, 2+, 3+) and percentage (0% to 100%) of claudin 18.2-stained tumor cells, calculating the histoscore (H-score) by multiplying the intensity score by the percentage.

Epstein-Barr virus (EBV)-encoded RNA in situ hybridization. As part of the diagnostic work-up, *in situ* hybridization of EBV-encoded RNA was performed on all surgically and endoscopically resected gastric cancer cases. For this purpose, we utilized the INFORM EBV-encoded RNA probe (Ventana Medical Systems) following the manufacturer's instructions to assess the EBV status of the samples. Each hybridization run included positive controls obtained from patients with EBV-positive nasopharyngeal carcinoma.

Microsatellite instability (MSI) testing. MSI testing was performed for all surgically or endoscopically resected gastric cancer cases during the diagnostic work-up. We conducted MSI polymerase chain reaction testing using five National Cancer Institute markers (BAT-26, BAT-25, D5S346, D17S250, and S2S123) to determine the MSI status of tumors. Both representative tumor tissues and matched normal tissues were utilized for MSI testing. We employed a DNA auto sequencer (ABI 3731 Genetic Analyzer; Thermo Fisher Scientific, Waltham, MA, USA) to analyze polymerase chain reaction products. According to the revised Bethesda Guidelines (20), tumors showing instability at two or more loci were classified as MSI-high, those with instability at one locus as MSI-low, and those with no instability as microsatellite stable.

Statistical analysis. We evaluated relationships between clinicopathological parameters using the chi-square test, student's *t*-test, and Kruskal-Wallis test. For parameters with an expected frequency of less than five, we employed Fisher's exact test. Correlation with the H-score was assessed using Pearson's correlation coefficient. A value of $p<0.05$ was considered significant in all tests. All statistical analyses were conducted using R software (version 4.3.2; R Foundation for Statistical Computing, Vienna, Austria).

Results

Study cohort. Detailed clinicopathologic characteristics of gastric tumors are presented in Table I. The mean age of the patients was 64.1 years (range=39-85 years). Of the total, 46 (68.7%) were male and 21 (21.3%) were female. Detailed clinicopathologic characteristics of other tumor types are presented in Table II. Pancreatic tumors comprised six ductal adenocarcinomas, two intraductal papillary mucinous neoplasms (IPMNs), and one IPMN with invasive carcinoma.

Claudin 18.2 expression in gastric tumor. Overall, claudin 18.2 expression was present in 61 out of 67 cases (91.0%). Claudin 18.2 positivity was detected in 47.8% (32/67) of the cohort. There were no significant differences in

Table I. *Clinicopathological characteristics of gastric tumors according to claudin 18.2 positivity.*

Variable		Overall population (n=67)	Claudin 18.2 expression status		*p*-Value
			Positive (n=32, 47.8%)	Negative (n=35, 52.1%)	
Age, years	Mean (range)	64.1 (39-85)	64.7 (48-85)	63.7 (39-84)	0.703
Sex, n (%)	Male	46	22 (47.8%)	24 (52.2%)	>0.99
	Female	21	10 (47.6%)	11 (52.4%)	
Sampling method, n (%)	Biopsy	6	4 (66.6%)	2 (33.4%)	0.414
	Surgery/ESD	61	28 (45.9%)	33 (54.1%)	
Tumor location, n (%)	Upper	7	4 (57.1%)	3 (42.9%)	0.148
	Middle	25	15 (60.0%)	10 (40.0%)	
	Lower	34	12 (35.3%)	22 (64.7%)	
	Other	1	1 (100.0%)	0 (0.0%)	
Tumor depth, n (%)	EGC	29	13 (44.8%)	16 (55.2%)	0.786
	AGC	33	17 (51.5%)	16 (48.5%)	
	N/A	5	2 (40.0%)	3 (60.0%)	
Gastric cancer type, n (%)	Intestinal	37	16 (43.2%)	21 (56.8%)	0.216
	PCC	15	7 (46.7%)	8 (53.3%)	
	MSI-high	8	3 (37.5%)	5 (62.5%)	
	EBVa	7	6 (85.7%)	1 (14.3%)	
Paired samples, n (%)	Primary	20 (50.0%)	9 (50.0%)	11 (50.0%)	
	Metastasis	20 (50.0%)	9 (50.0%)	11 (50.0%)	>0.99

AGC: Advanced gastric cancer; EBVa: Epstein-Barr virus-associated; EGC: early gastric cancer; ESD: endoscopic submucosal dissection; MSI: microsatellite instability; N/A: not available; PCC: poorly cohesive carcinoma.

clinicopathological parameters between patients with claudin 18.2-positive and those with claudin 18.2-negative gastric cancer (Table I). However, claudin 18.2-positive tumors tended to be frequently found in upper and middle thirds [4/7 (57.1%) and 15/25 (60.0%), respectively] compared to the lower third (12/34, 35.3%) (p=0.148). When gastric cancer cases were categorized into intestinal, poorly cohesive carcinoma, EBV-associated, and MSI-high subtypes similarly to The Cancer Genome Atlas classification (21), the frequency of claudin 18.2 positivity was higher in EBV-associated gastric cancer compared to other subtypes (6/7, 85.7%), although not statistically significantly (p=0.216). Claudin 18.2-positive cases in this subtype tended to show a diffuse and homogenous claudin 18.2 staining pattern. A heterogeneous claudin 18.2 staining pattern was occasionally observed in MSI-high and intestinal-type gastric cancer. Three intestinal-type adenocarcinomas had a focal neuroendocrine carcinoma component (less than 10%), which showed reduced to lack of claudin 18.2 staining (Figure 1). While there were no statistically significant differences in claudin 18.2 positivity among gastric cancer subtypes, the expression level (as measured by the H-score) differed significantly, with EBV-associated gastric cancer showing the highest expression (p=0.0167) (Figure 2A).

We also compared the claudin 18.2 expression pattern in paired primary and metastatic tumors. Generally, the claudin 18.2 expression levels of primary tumors and their metastasis showed a good correlation (r=0.84). However, we observed discrepancies in claudin 18.2 positivity between primary and metastatic tumors in four out of 20 cases (20%) (Figure 2B).

Claudin 18.2 expression in hepatobiliary and pancreatic tumors. Among all 17 hepatobiliary and pancreatic tumors, there was at least focal expression of claudin 18.2. However, claudin 18.2 positivity was observed in only four cases (two hepatobiliary tract cancer and two pancreatic IPMNs). Interestingly, invasive components and tumors within the lymphatic space tended to show low-level or a

Table II. *Clinicopathological characteristics of gastric tumors according to claudin 18.2 positivity.*

Tumor type		Overall (n=73)	Claudin 18.2 expression, n (%)		
			Any level	Positive (n=13)	Negative (n=60)
Hepatobiliary		8	8 (100.0%)	2 (25.0%)	6 (75.0%)
Pancreatic		9	9 (100.0%)	2 (22.2%)	7 (77.8%)
Ovary	Mucinous	5	5 (100.0%)	5 (100.0%)	0 (0.0%)
	HGSC	5	0 (0.0%)	0 (0.0%)	5 (100.0%)
Lung	Mucinous	5	5 (100.0%)	4 (80.0%)	1 (20.0%)
	Non-mucinous	5	4 (80.0%)	0 (0.0%)	5 (100.0%)
Uterine cervix	ADC, HPV-associated	4	0 (0.0%)	0 (0.0%)	4 (100.0%)
	ADC, gastric	4	4 (100.0%)	0 (0.0%)	4 (100.0%)
	SCC	3	0 (0.0%)	0 (0.0%)	3 (100.0%)
Endometrial		8	0 (0.0%)	0 (0.0%)	8 (100.0%)
Thyroid PTC		5	0 (0.0%)	0 (0.0%)	5 (100.0%)
Colonic		5	0 (0.0%)	0 (0.0%)	5 (100.0%)
Esophageal		2	0 (0.0%)	0 (0.0%)	2 (100.0%)
Lymphoma		4	0 (0.0%)	0 (0.0%)	4 (100.0%)
MUO		1	0 (0.0%)	0 (0.0%)	1 (100.0%)

ADC: Adenocarcinoma; HGSC: high-grade serous carcinoma; MUO: malignancy of unknown origin; PTC: papillary thyroid cancer; SCC: squamous cell carcinoma.

lack of claudin 18.2 staining. In contrast, IPMN and pancreatic intraepithelial neoplasia components frequently demonstrated diffuse and homogenous claudin 18.2 immunostaining (Table II and Figure 3). All IPMNs were gastric-type, based on histology.

*Claudin 18.2 expression in other tumor types. W*e also investigated claudin 18.2 expression in other tumor types. As expected, claudin 18.2 positivity was exclusively found in tumors showing gastric epithelium-like differentiation, including mucinous ovarian carcinoma (5/5, 100%) and invasive mucinous lung adenocarcinoma (4/5, 80.0%). None of the uterine cervix adenocarcinomas of the gastric type demonstrated claudin 18.2 positivity. However, expression of claudin 18.2 was found in all four cases (Table II and Figure 4).

Discussion

In this study, we conducted a comprehensive analysis of claudin 18.2 expression in gastric neoplasms and various other tumor types. Previous studies have reported claudin 18.2 expression status in different tumor types, but their clinicopathological significance was limited due to the use of various claudin 18 antibodies other than clone 43-14A. Additionally, different positive cut-off values further complicated the finding (10, 13, 22). Clone 43-14A, employed as a predictive biomarker for zolbetuximab efficacy in pivotal clinical trials, targets an epitope within the *C*-terminal domain of the protein (23). Claudin 18 exists in two isoforms: claudin 18.1 and claudin 18.2, which are nearly identical in amino acid sequence, differing by only 69 residues near the *N*-terminal domain (6, 10). Although clone 43-14A does not distinguish between claudin 18.1 and claudin 18.2, the latter isoform is predominantly expressed in normal and neoplastic gastric tissues. Consequently, this lack of specificity is not an issue in gastric cancer specimens (10, 23). However, using clone 43-14A may lead to false-positive results for claudin 18.2 expression in tumor types other than gastric cancer. In our study, this possibility was partly suggested by the finding that many non-mucinous lung adenocarcinomas showed focal and weak membranous claudin 18 immunostaining (Table II and Figure 4).

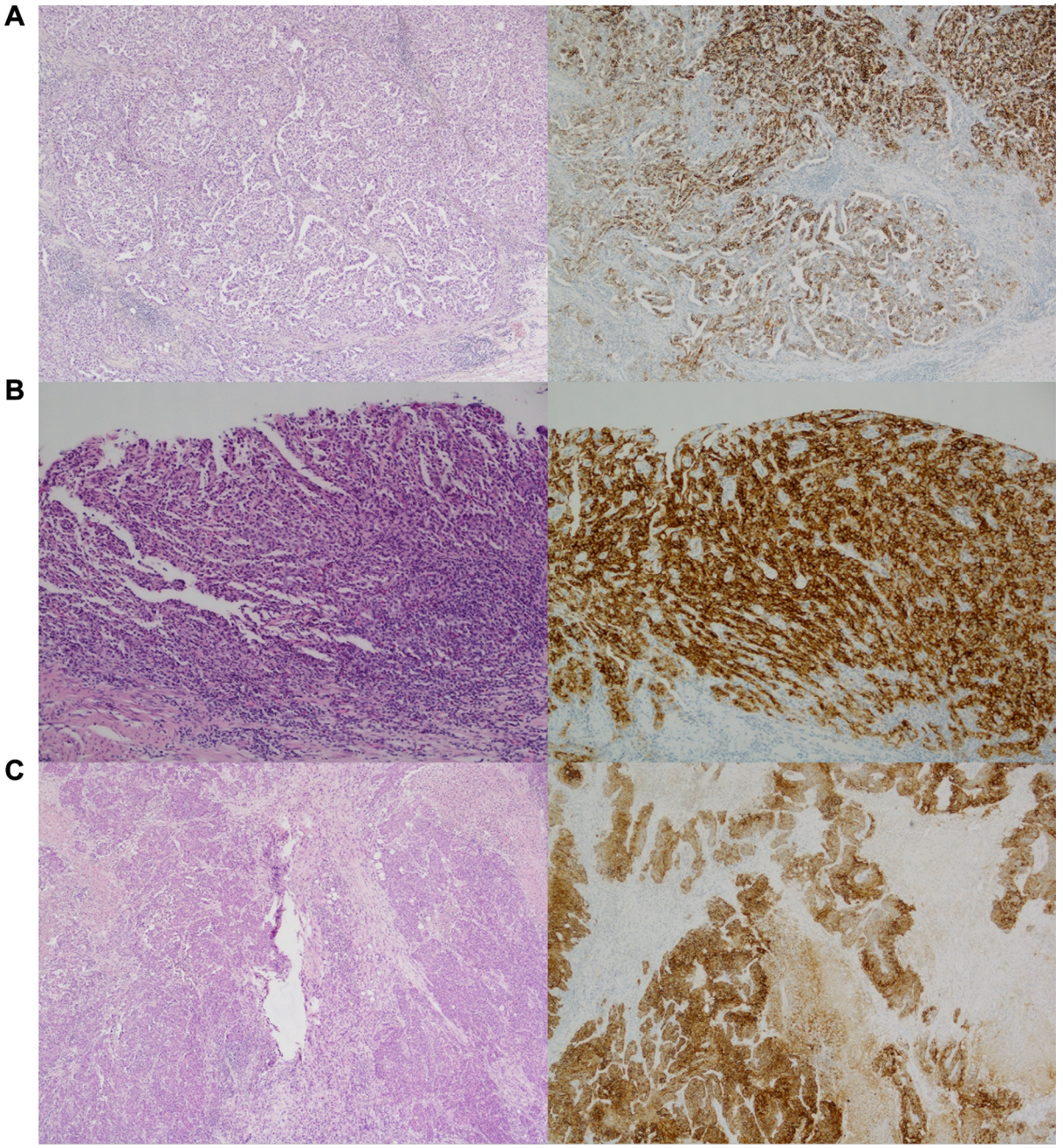

Figure 1. *Continued*

Despite this limitation, tumor types that frequently exhibited diffuse and strong claudin 18 expression in this study, such as invasive mucinous adenocarcinoma of the lung and mucinous carcinoma of the ovary, have been shown in several previous studies to express claudin 18.2 rather than claudin 18.1 (10, 13). Moreover, the gastric epithelium-like histology of these tumors further supports their expression of claudin 18.2 rather than claudin 18.1.

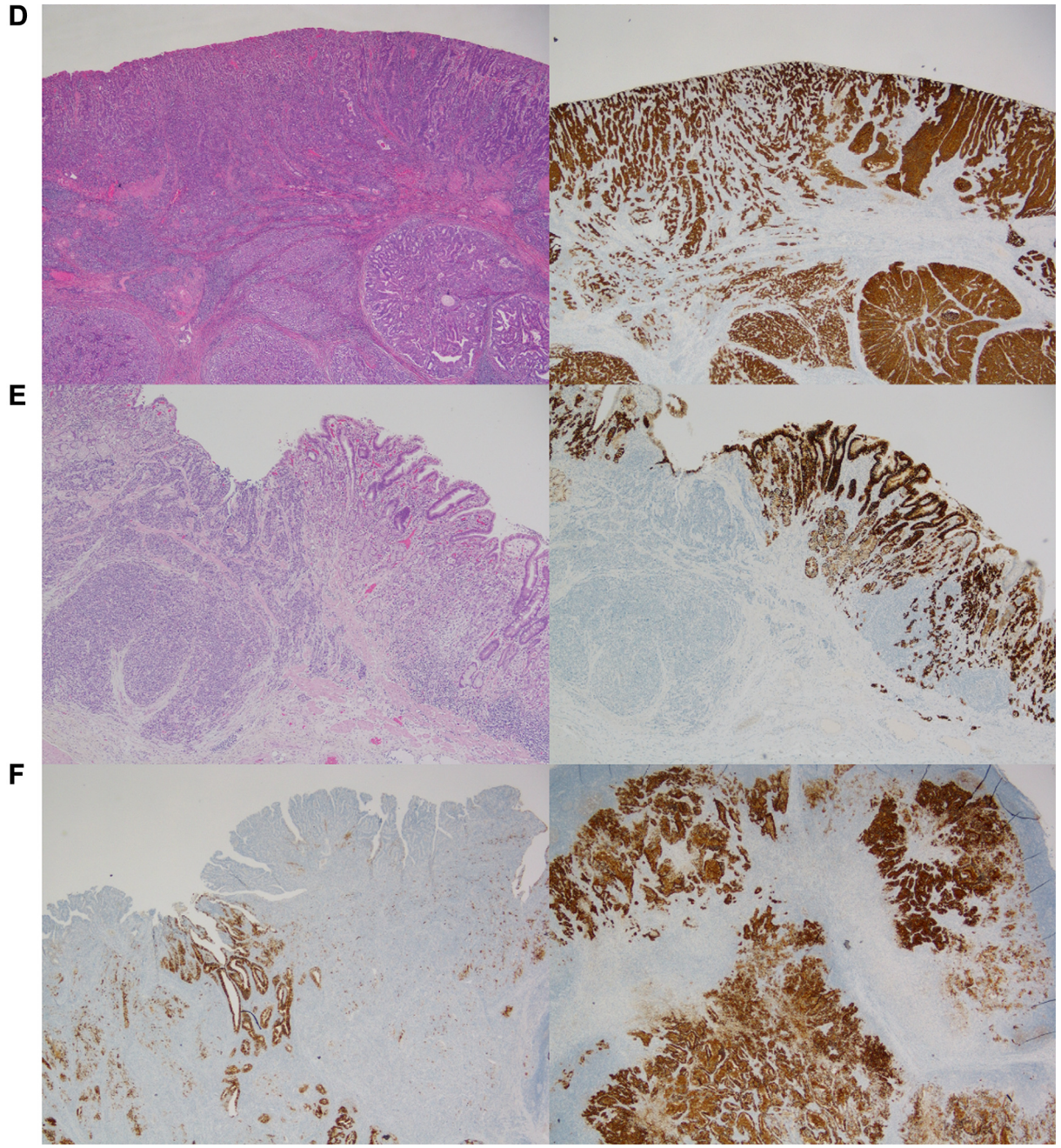

Figure 1. *Representative images of hematoxylin and eosin (HE)-stained (left panel) and claudin 18.2-immunostained (right panel) tumor tissue from different gastric cancer subtypes, along with an example of claudin 18.2-immunostained paired primary and lymph node metastasis. (A) Intestinal-type gastric cancer with heterogeneous claudin 18.2 staining (weak to strong membranous staining). (B) Poorly cohesive carcinoma. Diffuse and homogenous claudin 18.2 staining was found. (C) Microsatellite instability-high type gastric cancer, with heterogeneous claudin 18.2 staining (weak to strong membranous staining). (D) Epstein-Barr virus-associated gastric cancer, showing diffuse and homogenous claudin 18.2 staining. (E) Intestinal-type gastric cancer, with focal neuroendocrine differentiation (less than 10%). While the conventional adenocarcinoma component showed strong membranous staining, the small-cell neuroendocrine carcinoma component shows a complete absence of staining. (F) Primary gastric cancer (left) and its corresponding lymph node metastasis (right). While most tumor cells in the stomach were negative for claudin 18.2 immunostaining, metastatic tumor cells in the lymph node were claudin 18.2-positive. The tumor was microsatellite instability-high type. Original magnifications: A, C, E: ×40; B: ×100; C, D, F: ×5.*

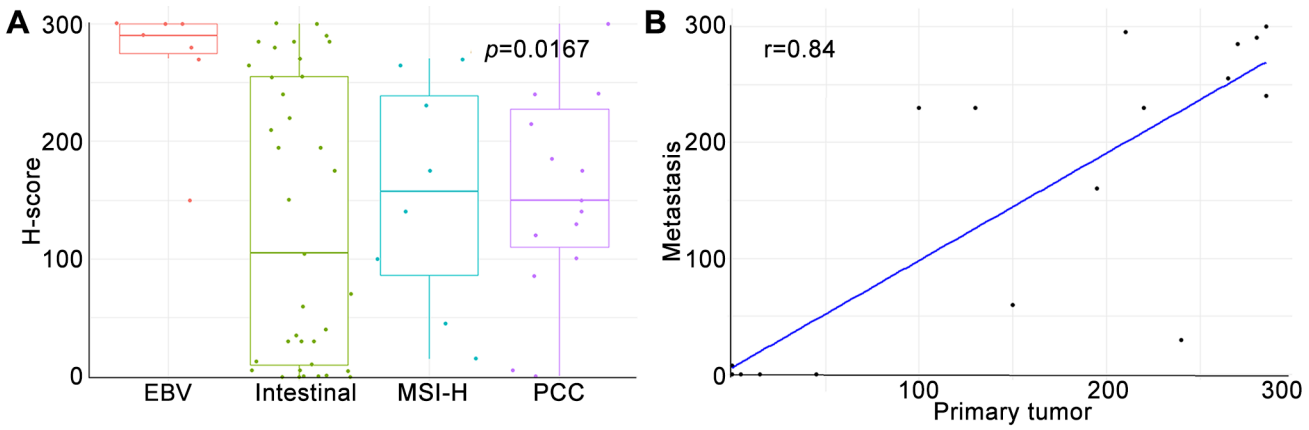

Figure 2. *(A) Boxplot showing H-scores for claudin 18.2 expression according to gastric tumor subtypes. Each box spans the 25th to 75th percentiles of claudin 18.2 H-scores. The line inside each box represents the median H-score. Bars extend from the box out to the minimum/maximum data points. Circles show the individual H-score for each sample (jittered horizontally). (B) Correlation of claudin 18.2 expression level (H-score) between primary tumors and their metastases. EBV: Epstein-Barr virus-associated gastric cancer; MSI-H: microsatellite instability-high; PCC: poorly cohesive carcinoma.*

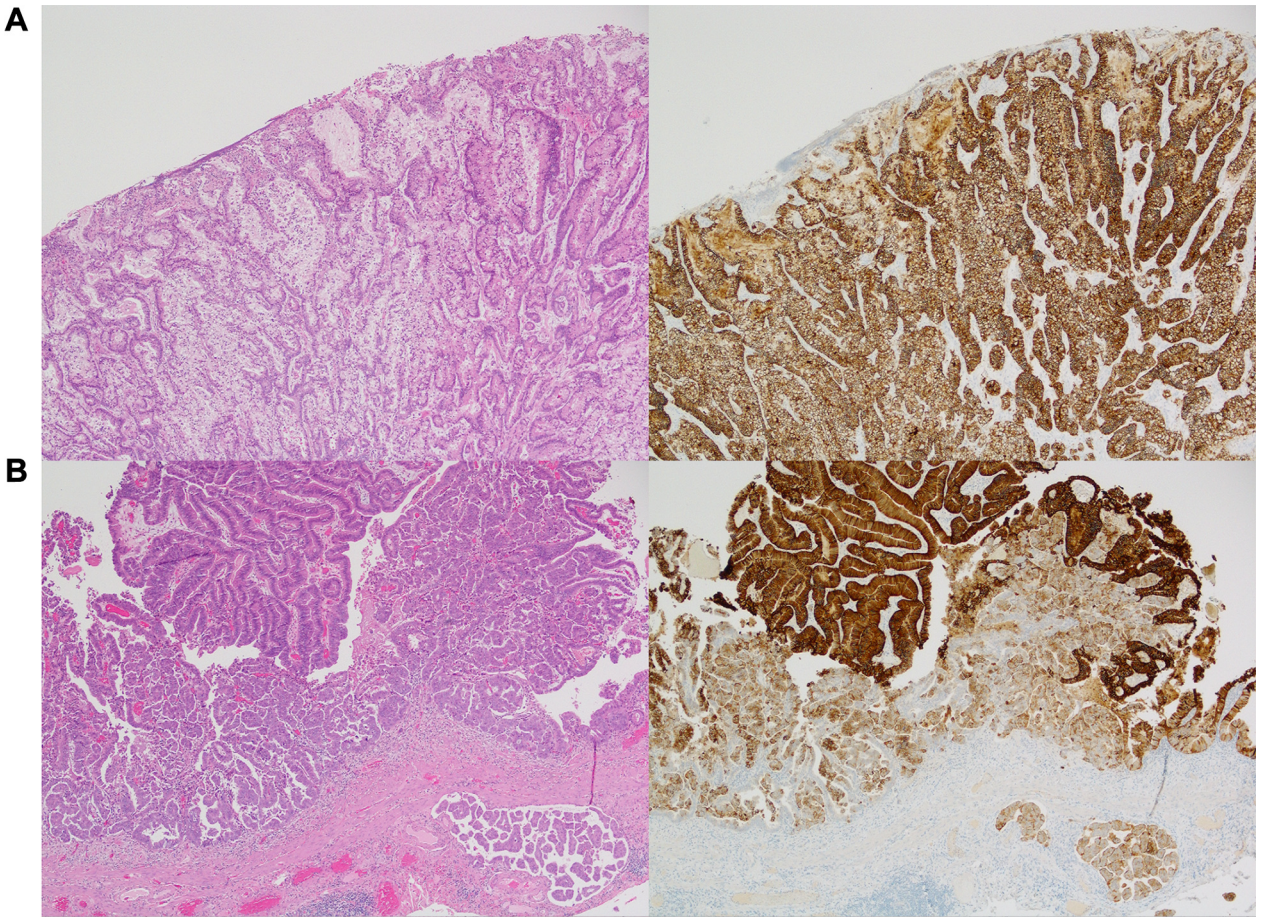

Figure 3. *Continued*

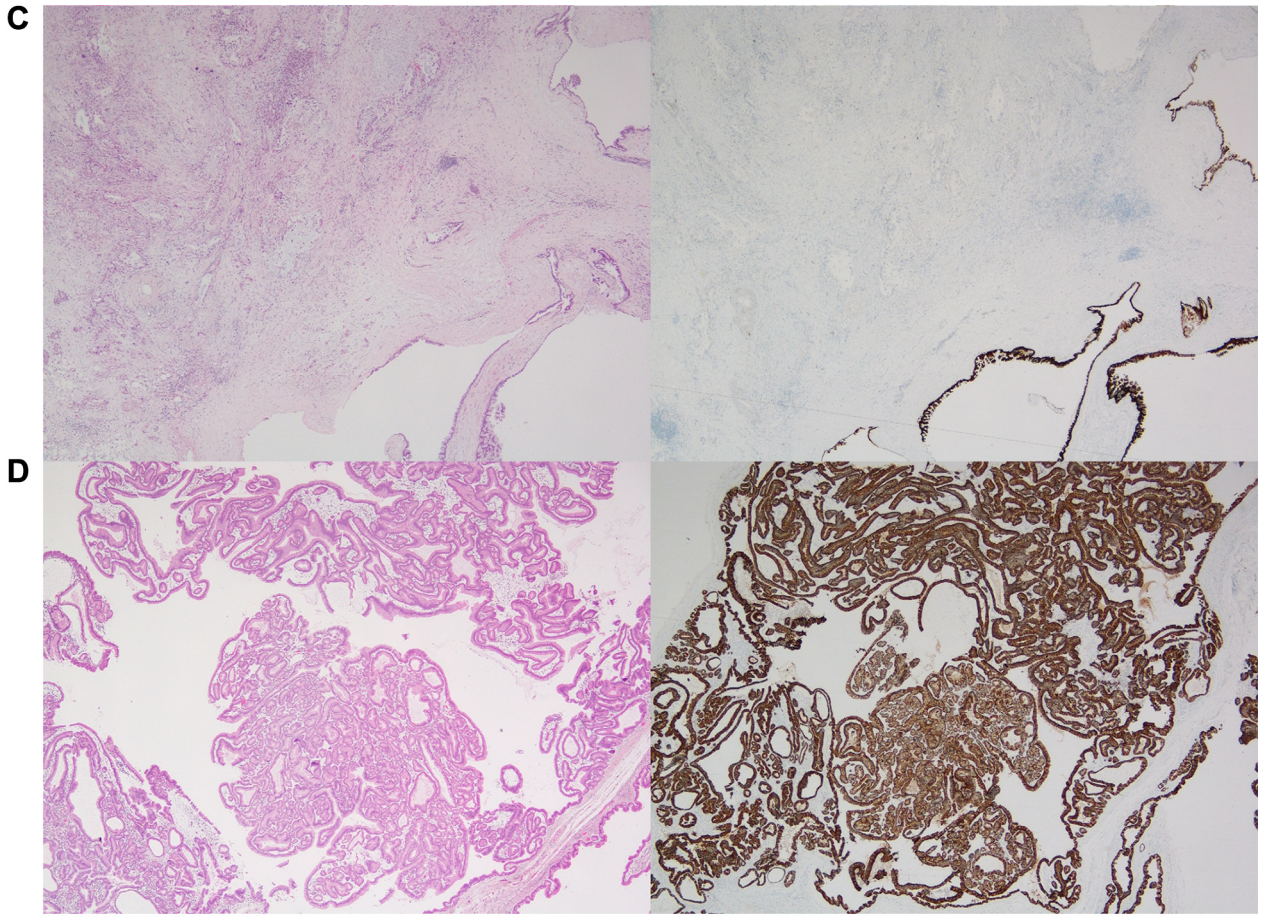

Figure 3. *Representative images of hematoxylin and eosin (HE)-stained (left panel) and claudin 18.2-immunostained (right panel) tumor tissue from hepatobiliary and pancreatic tumors. (A) Hepatobiliary cancer, with the majority of tumor cells showing moderate to strong membranous positivity for claudin 18.2. (B) Hepatobiliary cancer, with superficial tumor cells strongly positive for claudin 18.2, whilst tumor cells at the invasive front and lymphatic space showed a reduced staining intensity. (C) Pancreatic tumor. While the intraepithelial neoplasia component showed homogenous diffuse claudin 18.2 staining, the invasive component was negative for claudin 18.2 staining. (D) Intraductal papillary mucinous neoplasm of the pancreas. All the tumor cells showed diffuse homogenous staining. Original magnifications: A, B: ×40; C, D: ×5.*

In stomach tumors, claudin 18.2 positivity was not associated with most clinicopathological parameters. However, previous studies have consistently shown that claudin 18.2 positivity is more frequently found in diffuse-type gastric cancer than in the intestinal-type (11, 24). Additionally, some studies have reported a possible association between claudin 18.2 positivity and EBV-associated gastric cancer (25, 26), which the findings of this study align with. Although claudin 18.2 positivity did not significantly differ among gastric tumor subtypes in our cohort, this lack of significance may be attributed to the relatively small sample size. Indeed, the level of claudin 18.2 expression (measured by the H-score) did show significant variation among subtypes.

In gastric tumors with nodal or distant metastasis, the expression status of claudin 18.2 demonstrated a relatively good correlation, with only four discordant cases (4/20, 20.0%). Additionally, the expression levels of the primary tumor site and metastatic lesions also showed good correlation (r=0.84). Consequently, claudin 18.2 immunostaining can be reliably performed in metastatic

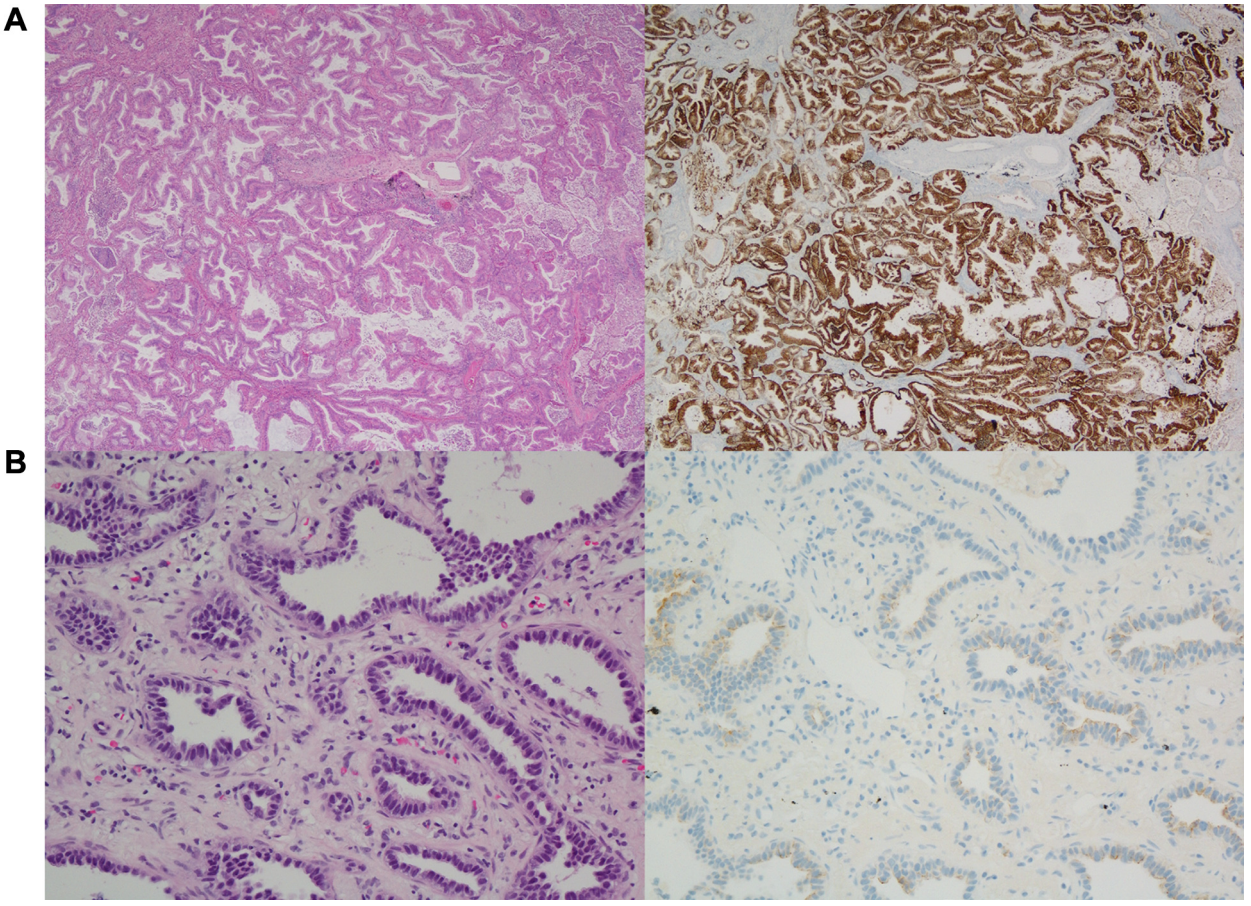

Figure 4. *Continued*

lesions and primary gastric tumors to identify patients suitable for targeted therapy.

Besides gastric tumors, claudin 18.2 positivity has been observed in hepatobiliary and pancreatic tumors, mucinous ovarian carcinoma, and invasive mucinous lung adenocarcinoma. Additionally, claudin 18.2 expression was found in uterine cervix adenocarcinoma of the gastric type. These claudin 18.2-expressing tumors are known to exhibit gastric-epithelium-like differentiation (27-29). Since many of these claudin 18.2-positive tumors currently lack actionable targets (*e.g.*, invasive mucinous lung adenocarcinoma and mucinous ovarian adenocarcinoma) (30, 31), claudin 18.2 might serve as a promising therapeutic target. Of note, pancreatic cancer, a potential candidate for claudin 18.2-targeted therapy, often showed reduced or no claudin 18.2 expression in the invasive component in this study. This might lead to a lower response to claudin 18.2-targeted therapy, although subsequent studies should be completed to verify the biological significance of this phenomenon.

Given that claudin 18.2 positivity and any level of expression were strongly associated with gastric origin or tumors with gastric epithelium-like differentiation ($p<0.001$), claudin 18.2 immunostaining can also be used as a lineage marker for gastric differentiation (Figure 5). It is particularly useful to differentiate upper from lower digestive tract cancer since claudin 18.2 is rarely expressed in lower digestive tract cancer (10, 13).

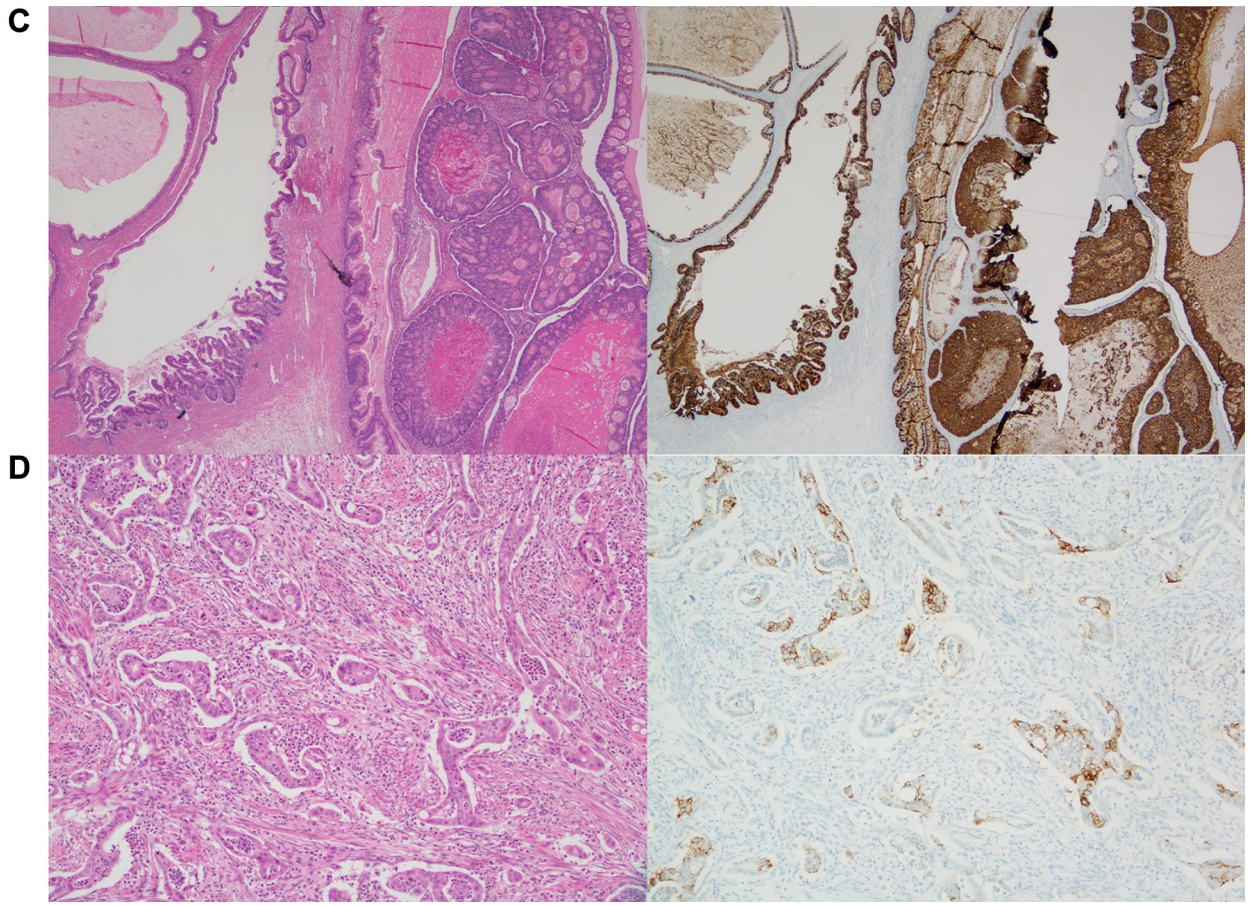

Figure 4. *Representative images of hematoxylin and eosin (HE)-stained (left panel) and claudin 18.2-immunostained (right panel) tumor tissue from other tumor types. (A) Invasive mucinous lung adenocarcinoma. Diffuse and strong membranous positivity was found in nearly all tumor cells. (B) Non-mucinous lung adenocarcinoma. Weak, apical staining was found in some tumor cells. (C) Mucinous ovarian tumor. All tumor components, from benign mucinous cystadenoma to malignant mucinous carcinoma, showed diffuse and strong membranous staining. (D) Uterine cervical adenocarcinoma, gastric type. Some tumor cells (less than 50%) showed heterogeneous claudin 18.2 staining (weak to strong membranous staining). Original magnifications: A, C: ×5; B: ×200; D: ×100.*

This study has some limitations. Firstly, relatively small tumor samples were used, particularly for tumor types other than gastric cancer. Subsequent studies using larger cohorts may be necessary to further strengthen the results. Secondly, due to the limited number of cases and a relatively short follow-up period, we did not perform overall or progression-free survival analyses. We also did not perform mucin immunostaining to further validate gastric epithelium-like differentiation in other tumor types. However, aberrant mucin expression patterns have been well-documented as being discordant with histological appearance in adenocarcinomas (32, 33). Therefore, we focused on histology to determine gastric differentiation in other tumor types.

Conclusion

Despite these limitations, this study represents the first investigation of claudin 18.2 expression across diverse tumor types using the same antibody and applying the same positivity cut-off criteria as major clinical trials, to the best of our knowledge. The study results show claudin

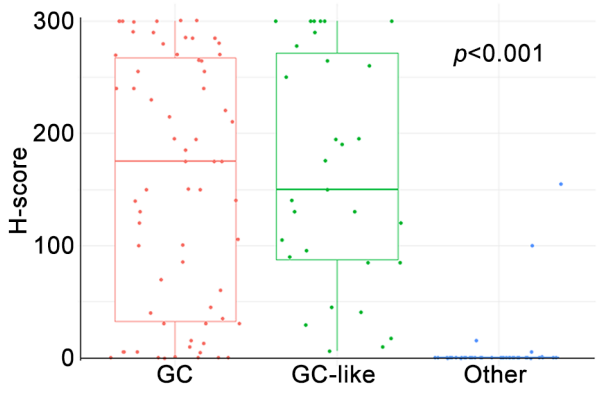

Figure 5. *Boxplot showing H-scores for claudin 18.2 expression according to gastric epithelium-like differentiation. Each box spans the 25th to 75th percentiles of claudin 18.2 H-scores. The line inside each box represents the median H-score. Bars extend from the box out to the minimum/maximum data points. Circles show the individual H-score for each sample (jittered horizontally). GC: Gastric cancer.*

18.2 expression status has utility for predicting which patients may be potential candidates for claudin 18.2 targeted therapy. Moreover, given the strong association between claudin 18.2 positivity and gastric epithelium-like differentiation, assessing claudin 18.2 expression status in all tumor types exhibiting gastric epithelium-like differentiation is advisable.

Funding

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Conflict of Interest

There are no conflicts or competing financial interests in relation to the work described here.

Authors' Contributions

ANS and MSK conceived and designed the study. MSK drafted the manuscript. MSK and HYW interpreted immunohistochemical staining. JHK collected and analyzed the clinicopathological data. JHK and HYW analyzed previous articles on claudin 18.2. JHK, ANS, and MSK reviewed and revised the manuscript carefully. All Authors read and approved the final manuscript.

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