BMC Cancer

RESEARCH

Open Access

Claudin 18.2 expression profile in primary tumors and their ovarian metastases: implications for targeted therapy

Nah Ihm Kim[1†], Joo Yeon Koo[1†], Sung Sun Kim[1], Ji Young Lee[1], Ji Shin Lee[1], Hyun Jin Bang[2], Woo Kyun Bae[2], Tae Mi Yoon[3], Kyung-Sub Moon[4], Jae-Hyuk Lee[1*] and Kyung-Hwa Lee[1,5*]

Abstract

Background Claudin 18.2 (CLDN18.2), a tight junction protein predominantly expressed in the normal gastric epithelium, has recently emerged as a potential therapeutic target in various solid tumors. Despite growing interest, comprehensive data on CLDN18.2 expression across primary tumors from different organs and their corresponding metastatic lesions remain limited.

Methods This study analyzed CLDN18.2 expression in 102 patients with primary adenocarcinomas from various organs and their corresponding ovarian metastatic carcinomas and in 81 cases of primary ovarian mucinous tumors using immunohistochemistry. We evaluated the association of CLDN18.2 expression with clinicopathologic features and survival outcomes.

Results The highest CLDN18.2 positivity rate was observed in gastric adenocarcinomas (40%, 12/30), followed by cervical adenocarcinomas (20%, 1/5) and colorectal adenocarcinomas (4%, 2/50). Notably, primary ovarian mucinous tumors showed remarkably high expression rates, reaching 77% overall and 100% in mucinous borderline tumors. In contrast, adenocarcinomas of the appendix and breast lacked CLDN18 expression. While CLDN18.2 expression was generally maintained during metastasis, some variations in expression patterns were observed, particularly in gastric cancers (13%, 4/30). Our analysis found no significant correlation between CLDN18.2 expression and overall survival in the patient cohort.

Conclusion The preserved expression of CLDN18.2 in metastatic tumors underscores its potential utility as a target for therapeutic approaches. Our findings emphasize the importance of evaluating CLDN18.2 status in both primary and metastatic tumors to refine therapeutic strategies.

Keywords Human CLDN18 protein, Adenocarcinoma, Neoplasm metastasis, Ovarian neoplasms, Survival analysis, Therapeutics

[†]Nah Ihm Kim and Joo Yeon Koo contributed equally to this work.

*Correspondence:
Jae-Hyuk Lee
jhlee@chonnam.ac.kr
Kyung-Hwa Lee
mdkaylee@jnu.ac.kr
Full list of author information is available at the end of the article

Introduction

Advancements of medical science have led to significant improvements in cancer treatment, yet therapeutic options remain limited for patients with inoperable or metastatic cancers. This limitation underpins the urgent need for novel therapeutic targets, making identification of such targets a pivotal aspect of precision medicine.

Claudins (CLDN) are a family of tight junction proteins involved in regulating permeability, maintaining barrier function, and preserving the polarity of epithelial layers [1]. These proteins are integral to the formation and maintenance of tight junctions, which are crucial for cellular integrity and function. To date, 27 CLDN isoforms have been identified [2]. Among these, CLDN 18 exists in two isoforms: CLDN 18.1, primarily expressed in normal alveolar epithelium, and CLDN18.2, predominantly found in normal gastric mucosal cells. During carcinogenesis, the disruption of tight junctions exposes CLDN18.2 epitopes, making them accessible for targeted monoclonal antibody therapies [3–6]. Zolbetuximab, a chimeric IgG1 monoclonal antibody targeting CLDN18.2, has demonstrated significant improvements in median survival and overall survival when used in combination with standard chemotherapy as a first-line treatment [6–8]. Despite these promising results, the majority of studies on CLDN18.2 expression have focused on primary tumors, with limited data on its expression in metastatic sites.

Given the dynamic nature of tumor biology, understanding the expression patterns of CLDN18.2 in both primary and metastatic tumors across various cancer types is crucial for optimizing targeted therapies. This study aims to analyze the expression of CLDN18.2 in a substantial cohort of patients with various primary tumors and their corresponding metastatic sites, with a particular focus on ovarian mucinous carcinomas. By employing immunohistochemical analysis, we seek to evaluate the potential of CLDN18.2 as a therapeutic target and biomarker across multiple cancer types and metastatic tumors.

Materials and methods

Patient cohort and clinicopathologic data

This study encompassed 102 patients with matched primary carcinoma and ovarian metastases, alongside 81 patients diagnosed with primary ovarian mucinous tumors. All patients underwent surgical resection of primary and metastatic lesions at Chonnam National University Hwasun Hospital between 2005 and 2024. Clinical data were retrospectively extracted from the patients' medical records, including age, primary tumor site, T stage, lymph node metastasis, and chemotherapy regimen. Pathologic staging adhered to the American Joint Committee on Cancer (AJCC) staging system, 8th edition [9]. Overall survival (OS) was calculated from the date of surgery until death or the last follow-up visit.

Formalin-fixed paraffin-embedded (FFPE) tumor blocks were retrieved from the archives of the Department of Pathology. Hematoxylin and eosin (H&E) slides were prepared for each case. Tumor sections were carefully selected from representative areas, with focus on identifying the most malignant and advanced foci. Two pathologists (KNI and LKH) independently reviewed the slides to evaluate histologic features. Tumors were classified and subtyped according to the World Health Organization classification of tumors of the female reproductive organs [12] and the digestive system [13]. Tumor-Node-Metastasis (TNM) staging was performed for the entire cohort according to the 8th edition of the AJCC staging system. Histopathologic evaluation included histologic type, histologic grade, and presence of lymphovascular invasion. Clinicopathological variables were consolidated into meaningful categories to facilitate statistical analysis. Well-differentiated and moderately differentiated tumors were categorized as low grade, while poorly differentiated tumors were classified as high grade. T stages 1 and 2 were grouped as low stage, while stages 3 and 4 were considered high stage. This study received approval from the Institutional Review Board of the Chonnam National University Hwasun Hospital (CNUHH-2024–108).

Claudin18 immunohistochemistry

Claudin18 (CLDN18) expression was assessed using immunohistochemistry (IHC) with CLDN18 antibody (Clone 43-14A, Roche Diagnostics, Rotkreuz, Switzerland). Tissue sections. (3 μm thick) from paraffin-embedded tissue blocks were stained using an automated immunostainer (BenchMark ULTRA, Roche Diagnostics) with the OptiView DAB IHC Detection Kit (Ventana Medical Systems, Tucson, AZ, USA), following the manufacturer's protocol. Sections were pretreated with ULTRA Cell Conditioning Solution (ULTRA CC1, tris–EDTA buffer pH 7.8) before incubation with CLDN18 antibody. Human gastric mucosa tissue served as a positive control.

Immunohistochemistry scoring

Two experienced pathologists (KNI and LKH), blinded to the clinical data, independently assessed the IHC staining using criteria based on the phase III SPOTLIGHT biomarker study criteria [6, 8]. Assessment was performed manually using a microscope, focusing on cell membrane staining. The percentage of positive tumor cells and staining intensity were evaluated, with intensity categorized as 0 (no staining), 1 (weak), 2 (moderate), or 3 (strong). CLDN18 positivity was defined as $\geq$75% in

tumor cells exhibiting moderate-to-strong membranous staining. Inter-observer agreement of immunohistochemical assessment between the two pathologists was evaluated using Cohen's kappa coefficient (κ). Analysis demonstrated high concordance (κ = 0.921), confirming strong reliability in CLDN18.2 immunohistochemical staining interpretation.

Statistical analysis

Statistical analysis was conducted using SPSS Statistics, version 29.0 (IBM Corp., Armonk, NY, USA) for Windows. Relationships between CLDN18 expression and clinicopathologic parameters were evaluated using Pearson chi-squared test or Fisher's exact test as appropriate. Inter-observer agreement for immunohistochemical assessment between pathologists was evaluated using Cohen's kappa coefficient. Univariate and multivariate analyses, including Cox proportional hazard models, were employed to determine the effects of individual variables on survival and identify independent prognostic factors.

Overall survival was estimated using the Kaplan–Meier method, with between-group differences assessed by a stratified log-rank test. A Cox proportional hazard model, stratified by age, CLDN18 positivity in primary, primary organs, histologic grade, T stage, lymph node metastasis, lymphovascular invasion, and chemotherapy regimen, was used to estimate hazard ratios (HRs) and corresponding 95% confidence intervals (CIs). Statistical significance was set at $p < 0.05$.

Results

Characteristic of the patients

This study examined 102 patients with adenocarcinoma originating from various organs and matched ovarian metastatic carcinoma. The patients had a mean age of 52.1 years. The primary tumors predominantly arose from the gastrointestinal tract (90/102, 88%), with the majority coming from the colorectum (50/102, 49%), followed by the stomach (30/102, 29%) and appendix (10/102, 10%). Non-gastrointestinal malignancies included breast carcinoma (7/102, 7%) and cervical adenocarcinoma (5/102, 5%). HPV genotyping of the cervical adenocarcinomas revealed all five cases were positive for high-risk HPV: three with HPV16, one with HPV18, and one with both HPV59 and HPV66. Histologically, the tumors showed a slight predominance of low-grade tumors (56/102, 55%). Most patients presented with high T stage (T3 or T4) according to the AJCC criteria (86/102, 84%), and a significant proportion had lymph node metastasis (73/102, 72%). Approximately half of the patients had lymphovascular invasion (52/102, 51%). Following diagnosis, the majority of the patients underwent platinum-based chemotherapy (77/102, 75%).

Additionally, the study included 81 patients with primary ovarian mucinous tumors, who had a mean age of 49.4 years. These cases were predominantly mucinous carcinomas, with a small number of mucinous borderline tumors (12/81, 15%). In contrast to the metastatic group, most of these tumors were histologically low-grade (74/81, 91%) and had a low T stage (74/81, 91%). Lymph node metastasis and lymphovascular invasion were rare in this group (2 cases and 4 cases, respectively). Forty-four patients received platinum-based chemotherapy after surgery and histological diagnosis.

Correlation between CLDN18 expression and clinicopathologic variables

The expression of CLDN18 varied significantly across different primary organs (Table 1). Gastric adenocarcinomas showed the highest positivity rate (12/30, 40%), followed by those of the uterine cervix (1/5, 20%) and colorectum (2/50, 4%) ($P < 0.001$). Notably, adenocarcinomas of the appendix and breast showed no CLDN18 expression. CLDN18 expression tended to increase in histologically higher-grade tumors with statistical significance, while the CLDN18-positive cases tended to decrease in higher T stages ($P = 0.024$ and $P = 0.042$, respectively). However, no significant correlations were found between CLDN18 expression and other factors such as age, lymph node metastasis, or lymphovascular invasion ($P = 0.245$, $P = 0.546$, and $P = 0.411$, respectively). The chemotherapy regimen was not included in the evaluation of its association with CLDN expression, as it is a factor determined retrospectively after surgery and tissue diagnosis.

In the primary ovarian mucinous tumor group, CLDN expression positivity was notably high (62/81, 77%), particularly in mucinous borderline tumors where all cases were positive (12/12, 100%) ($P = 0.038$). However, no significant correlations were found between CLDN expression and clinicopathologic variables such as age, histological grade, T stage, lymph node metastasis, or lymphovascular invasion in this group ($P = 0.631$, $P = 0.664$, $P = 0.205$, $P = 0.053$, and $P = 0.568$, respectively) (Table 2).

Comparison of CLDN18 expression in pairs of primary and metastatic carcinomas

This study examined 102 paired samples of primary adenocarcinomas and their corresponding ovarian metastases to assess CLDN18 expression. The analysis revealed cases where the expression status of CLDN18 changed

Table 1 Correlation between CLDN18 expression and clinicopathologic variables of 102 patients with ovarian metastasis

Clinicopathologic variables		No	CLDN18 in primary organs		*P* value
			Negative	Positive	
Age (mean 52.1)	≤ 55 yr	65	53 (81%)	12 (19%)	0.245
	> 55 yr	37	34 (92%)	3 (8%)	
Primary organs	Stomach	30	18 (60%)	12 (40%)	< 0.001
	Appendix	10	10 (100%)	0	
	Breast	7	7 (100%)	0	
	Uterine cervix	5	4 (80%)	1 (20%)	
	Colorectum	50	48 (96%)	2 (4%)	
Histological grade	Low-grade	56	52 (93%)	4 (7%)	0.024
	High-grade	46	35 (76%)	11 (24%)	
T stage	Low (T1 or T2)	16	11 (69%)	5 (31%)	0.042
	High (T3 or T4)	86	76 (88%)	10 (12%)	
Lymph node metastasis	Absent	29	26 (90%)	3 (10%)	0.546
	Present	73	61 (84%)	12 (16%)	
Lymphovascular invasion	Absent	50	41 (82%)	9 (18%)	0.411
	Present	52	46 (89%)	4 (11%)	
Chemotherapy	Platinum-based	77	66 (86%)	11(14%)	NA*
	Taxane-based	11	11 (100%)	0	
	Etc	14	10 (71%)	4 (29%)	
Total		102	87 (85%)	15 (15%)	

* *Abbreviation*: *NA* Not applicable

Table 2 Correlation between CLDN18 expression and clinicopathologic variables in 81 patients with ovarian mucinous tumor

Clinicopathologic variables		No	CLDN18 in primary organs		*P* value
			Negative	Positive	
Age (mean 49.4)	≤ 50 yr	38	8 (21%)	30 (79%)	0.631
	> 50 yr	43	11 (26%)	32 (74%)	
Mucinous tumor	Borderline	12	0 (0%)	12 (100%)	0.038
	Malignant	69	19 (27%)	50 (73%)	
Histological grade	Low-grade	74	17 (23%)	57 (77%)	0.664
	High-grade	7	2 (29%)	5 (71%)	
T stage	Low (T1 or T2)	74	16 (22%)	58 (78%)	0.205
	High (T3 or T4)	7	3 (43%)	4 (57%)	
Lymph node metastasis	Absent	79	17 (21%)	62 (78%)	0.053
	Present	2	2 (100%)	0 (0%)	
Lymphovascular invasion	Absent	77	19 (25%)	58 (75%)	0.568
	Present	4	0 (0%)	4 (100%)	
Chemotherapy	None	37	3 (8%)	34 (92%)	NA*
	Platinum + Taxane	44	16 (36%)	28 (64%)	
Total		81	19 (23%)	62 (77%)	

* *Abbreviation*: *NA* Not applicable

between the primary and metastatic sites. Pairs were considered concordant when CLDN18 positivity was consistent in both the primary and metastatic sites. The analysis revealed that the majority of pairs were concordant (93/102, 91%), with a small fraction showing discordant expression patterns (9/102, 9%).

Expression status changes were most frequently observed in gastric cancer cases, which also exhibited the highest positive expression rate among various primary tumors (Table 3). In gastric cancer, both negative-to-positive and positive-to-negative conversions were observed. Specifically, there were three instances of negative-to-positive conversion and one case of positive-to-negative conversion (Fig. 1). Similar conversion patterns were noted in adenocarcinomas of other organs. Appendiceal and uterine cervix adenocarcinomas showed cases of negative-to-positive conversion, while colorectal adenocarcinoma exhibited cases of positive-to-negative conversion (Fig. 2).

Survival outcomes according to CLDN18 overexpression

The impact of various clinicopathologic variables, including CLDN18 expression, on patient overall survival was analyzed using the Kaplan–Meier method. CLDN18 expression, whether in the primary organ or ovarian metastatic lesions, did not significantly affect overall survival ($P=0.919$, Fig. 3A). However, several other factors were found to influence survival outcomes. Among the clinicopathologic variables examined, lymph node metastasis and lymphovascular invasion were associated with significantly reduced overall survival periods ($P=0.004$ and $P=0.008$, respectively, Fig. 3B-C). High-grade histology also showed a marginally significant decrease in survival periods ($P=0.076$). Multivariate analysis using the Cox proportional hazards model revealed that primary organ, histologic grade, lymph node metastasis, and lymphovascular invasion were independent prognostic factors for overall survival ($P<0.001$, $P<0.001$, $P=0.001$, and $P=0.005$, respectively) (Table 4).

In the primary ovarian mucinous tumor group, CLDN18 positivity similarly showed no significant relationship with the patients' overall survival ($P=0.399$, Fig. 3D, Table 5). Among various clinicopathologic variables in this group, high T stage group was associated with a significant decrease in overall survival compared to low T stage group ($P<0.001$, Fig. 3E). Lymphovascular invasion and chemotherapy showed marginal significance in affecting survival ($P=0.087$, and $P=0.054$, respectively, Fig. 3F). Multivariate analysis identified T stage as the sole independent prognostic factor ($P<0.001$) in this group.

Table 3 Conversion of CLDN18 expression status between primary cancer and ovarian metastasis

CLDN18 in primary organs		No	CLDN18 in ovarian metastasis		Kappa value
			Negative	Positive	
Stomach	Negative	18	15 (83%)	3 (17%)	0.730
	Positive	12	1 (8%)	11 (92%)	
Appendix	Negative	10	8 (80%)	2 (20%)	NA
	Positive	0	0	0	
Breast	Negative	7	7 (100%)	0	NA
	Positive	0	0	0	
Uterine cervix	Negative	4	3 (75%)	1 (25%)	0.545
	Positive	1	0	1 (100%)	
Colorectum	Negative	48	48 (100%)	0	NA
	Positive	2	2 (100%)	0	
Total	Negative	87	81 (93%)	6 (7%)	0.675
	Positive	15	3 (20%)	12 (80%)	
Sum		102	84	18	

Discussion

Advancements in medical science have enabled effective treatments for numerous cancer patients. However, therapeutic options, particularly chemotherapy, remain limited for individuals with inoperable cancer or metastasis. Consequently, the identification of novel therapeutic targets has become a crucial focus in precision medicine. In recent years, CLDN18.2 has emerged as a promising novel agent molecule [3–5, 12, 13]. Despite this progress, the majority of data on CLDN18.2 expression is predominantly confined to primary cancers, with a particular emphasis on gastric and pancreatic malignancies [12, 13, 12, 13, 12, 13, 12, 13]. Notably, there is a current lack of studies comparing CLDN18.2 expression between primary tumors in various organs and their corresponding metastatic tumors. To address this gap in knowledge, our study aimed to analyze CLDN18.2 expression in diverse primary tumors and their matched metastatic ovarian tumors using immunohistochemistry. Furthermore, we sought to evaluate the potential value of CLDN18.2 as a therapeutic target in these contexts.

Claudins (CLDN) are integral components of the tight junction family, which encodes two isoforms, CLDN18.1 and CLDN18.2, resulting from alternative splicing of exon. These isoforms share a high degree of homology but exhibit tissue-specific expression patterns. CLDN18.1 is primarily expressed in normal lung tissue and lung adenocarcinomas, while CLDN18.2 exhibits selective expression on the surface of differentiated epithelial cells in normal gastric mucosa [3, 12]. Notably, CLDN18.2 overexpression has been reported in malignancies of gastric, pancreatic, and esophageal origin, highlighting its potential role in oncogenesis. During the process of malignant transformation, tight junctions undergo disruption, leading to the exposure of the CLDN18.2 epitope on the surface of tumor cells [5]. While immunohistochemical staining for CLDN18 cannot distinguish between these

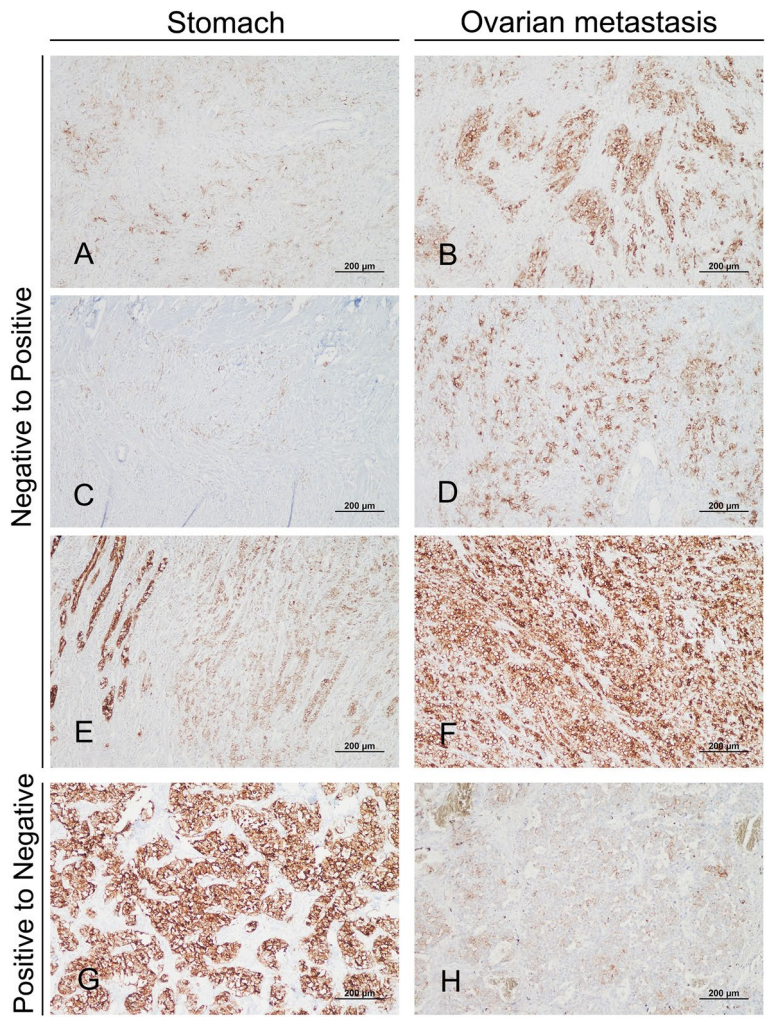

Fig. 1 Representative matched pairs illustrating conversion of CLDN18.2 expression. **A** through **F** Negative-to-positive conversion from stomach to ovarian metastasis; Case 35 with gastric primary of (2+, 30%, **A**) and ovarian metastasis of (2+, 80%, **B**), Case 36 with gastric primary of (1+, 30%, **C**) and ovarian metastasis of (2+, 80%, **D**), and Case 43 with gastric primary of (2+, 40%, **E**) and ovarian metastasis of (3+, 80%, **F**). (**G** and **H**) Positive-to-negative conversion from stomach to ovarian metastasis; Case 64 with gastric primary of (3+, 80%, **G**) and ovarian metastasis of (2+, 30%, **H**) (magnification × 200)

isoforms, the tissue-specific expression of CLDN18.2 allows for its clinical application as a biomarker and therapeutic target, particularly in cancers of gastric origin. Recent research, including phase III studies such as SPOTLIGHT and GLOW, has demonstrated that monoclonal antibody therapy targeting CLDN18.2 significantly improves overall survival in patients with unresectable gastric and esophageal adenocarcinomas [12, 13]. These promising findings have prompted further investigations into CLDN18.2 expression across various organs [13, 12]. Our results largely corroborate previously reported CLDN18.2 expression patterns while also revealing novel insights. Notably, the expression rate of CLDN18.2 exhibits geographic variations. A study utilizing the same monoclonal antibody (clone 43–14A) reported a positive rate of 53.0% in cases with gastric or esophageal adenocarcinoma in Germany [13], compared to 87.0% in Japan [12]. In our study, we observed a CLDN18.2 expression rate of 40% in both primary and metastatic gastric cancers, providing evidence that CLDN18.2 expression is maintained during the metastatic process.

Yan et al. conducted a study on CLDN18.2 expression in adenocarcinomas originating from various organs. Their findings revealed CLDN18.2 expression in lung mucinous adenocarcinoma, cholangiocarcinoma, colorectal mucinous adenocarcinoma, and gastric-type cervical adenocarcinoma. However, breast adenocarcinoma did not exhibit CLDN18.2 expression [12]. Our study

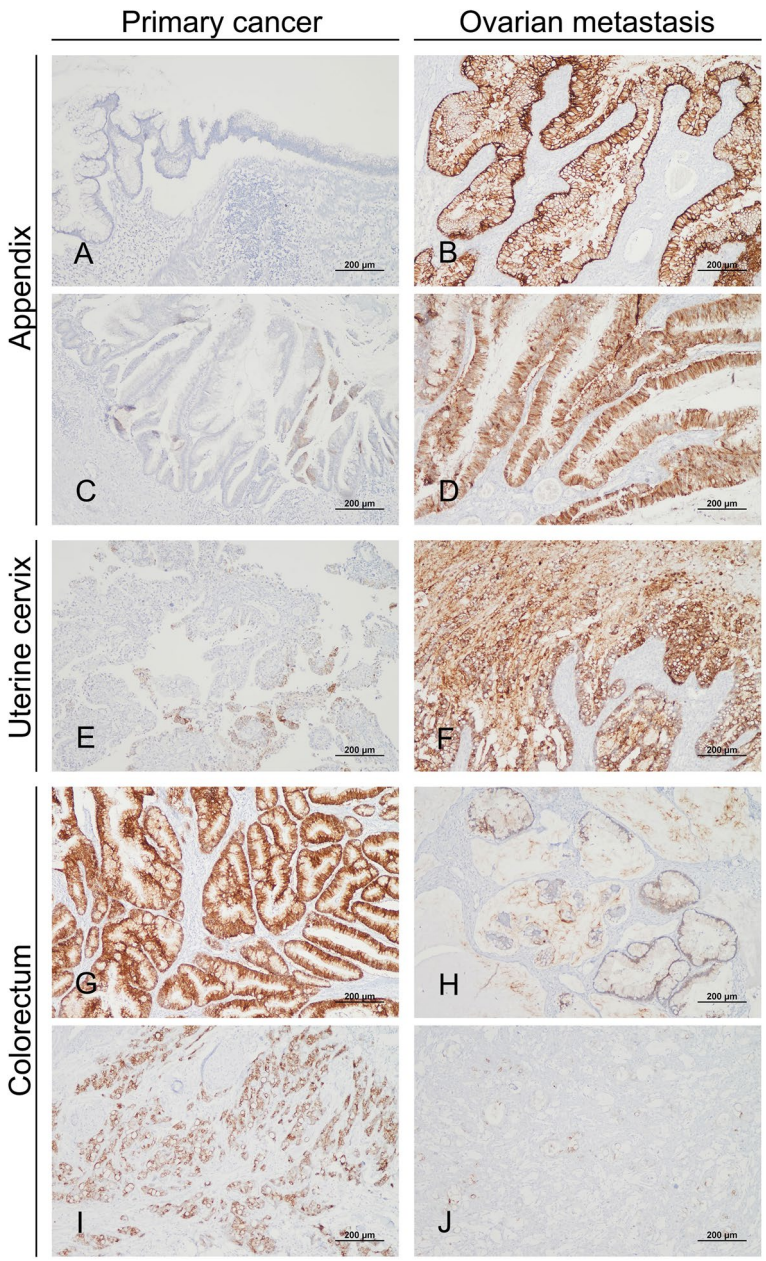

Fig. 2 Representative matched pairs illustrating conversion of CLDN18.2 expression. **A** through **F** Negative-to-positive conversion from primary tumor to ovarian metastasis; Case 40 with appendiceal primary of (0, 0%, **A**) and ovarian metastasis of (3 + , 90%, **B**), Case 154 with appendiceal primary of (2 + , 20%, **C**) and ovarian metastasis of (3 + , 80%, **D**), and Case 50 with uterine cervix primary of (2 + , 40%, **E**) and ovarian metastasis of (2 + , 80%, **F**). (**G** through **J**) Positive-to-negative conversion from primary tumor to ovarian metastasis; Case 38 with colorectal primary of (3 + , 80%, G) and ovarian metastasis of (1 + , 80%, **H**), and Case 145 with colorectal primary of (2 + , 80%, **I**) and ovarian metastasis of (2 + , 5%, **J**) (magnification × 200)

corroborated these results, confirming the absence of CLDN18.2 expression in breast cancer. Furthermore, we observed a high frequency of CLDN18.2 expression in ovarian mucinous tumors, aligning with the findings reported by Wagner et al. [13]. In their comprehensive analysis of primary ovarian cancer subtypes (serous, endometrioid, clear cell, and carcinosarcoma), Wagner et al. demonstrated that 99.5% of non-mucinous tumors lacked CLDN18.2 expression, with positivity almost exclusively limited to the mucinous subtype. Our results corroborate this observation, further substantiating the specific association between CLDN18.2 expression and

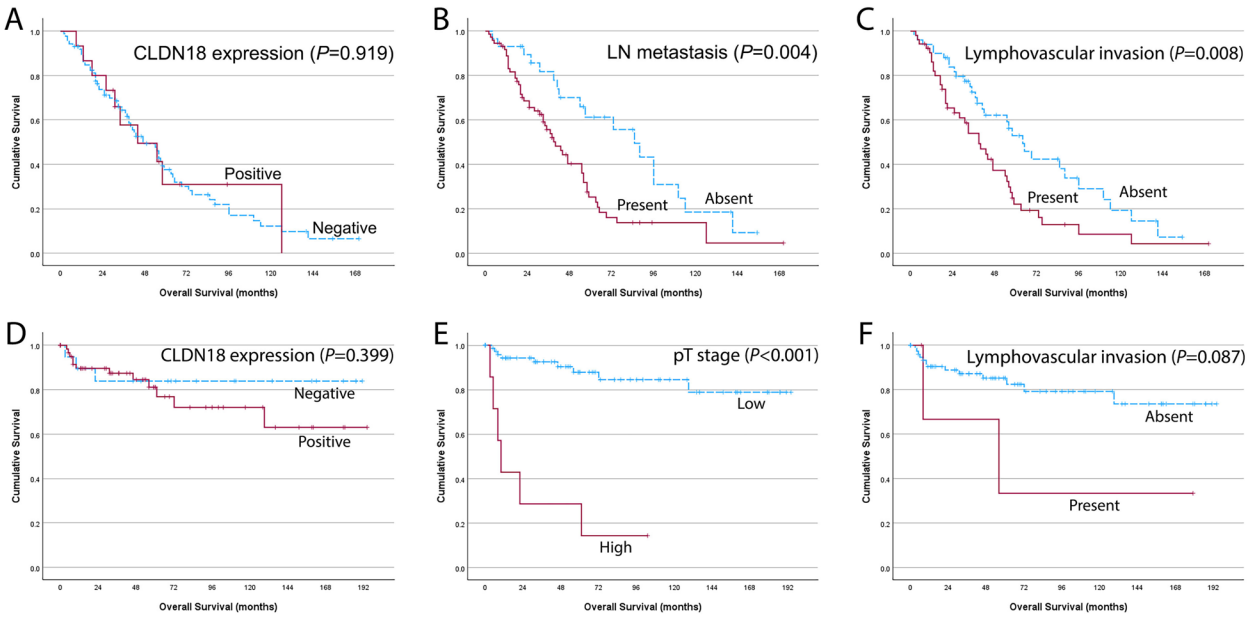

Fig. 3 Kaplan–Meier estimates of overall survival according to CLDN18 expression (**A**), lymph node metastasis (**B**), and lymphovascular invasion (**C**) in primary cancers with ovarian metastasis; and CLDN18 expression (**D**), pT stage (**E**), and lymphovascular invasion (**F**) in primary ovarian mucinous neoplasms

Table 4 Univariate and multivariate analysis for overall survival predictors in 102 patients with ovarian metastasis

Clinicopathologic variables		No	Mean survival (months)	P-value (univariate)	*P*-value (multivariate)†	Hazard ratio	95% confidence interval	
Age (mean 52.1)	≤ 55 yr	65	62.6	0.472	0.051	1	0.998	3.248
	> 55 yr	37	54.8			1.8		
CLDN18 in primary	Negative	87	59.5	0.919	0.578	1	0.564	2.973
	Positive	15	62.6			1.255		
Primary organs	Stomach	30	63.6	0.602	< 0.001	1		
	Appendix	10	78.1		0.007	5.934	1.611	21.863
	Breast	7	81.2		0.186	2.325	0.667	8.109
	Uterine cervix	5	63.2		0.007	10.092	1.881	54.134
	Colorectum	50	50.4		< 0.001	7.633	3.101	18.789
Histological grade	Low-grade	56	67.7	0.076	< 0.001	1	2.396	9.997
	High-grade	46	52.4			4.894		
T stage	Low (T1 or T2)	16	73.0	0.237	0.784	1	0.317	2.379
	High (T3 or T4)	86	56.5			0.869		
Lymph node metastasis	Absent	29	81.0	0.004	0.001	1	1.770	10.440
	Present	73	50.4			4.298		
Lymphovascular invasion	Absent	50	72.2	0.008	0.005	1	1.327	5.024
	Present	52	47.2			2.582		
Chemotherapy	Platinum-based	77	54.3	0.466	0.081	1		
	Taxane-based	11	69.5		0.330	0.634	0.254	1.585
	Etc	14	59.9		0.093	2.105	0.884	5.014

† Cox proportional hazards model for multivariate analysis

Table 5 Univariate and multivariate analysis for overall survival predictors in in 81 patients with ovarian mucinous tumor

Clinicopathologic variables		No	Mean survival (months)	P-value (univariate)	*P*-value (multivariate)†	Hazard ratio	95% confidence interval	
Age (mean 49.4)	≤ 50 yr	38	168.3	0.135	0.319	1	0.515	7.638
	> 50 yr	43	124.1			1.984		
CLDN18 ovary	Negative	19	162.1	0.399	0.499	1	0.415	6.075
	Positive	62	144.6			1.588		
Mucinous tumor	Borderline	12	145.3	0.550	0.587	1	0.029	7.472
	Malignant	69	151.0			0.463		
Histological grade	Low-grade	74	154.6	0.405	0.186	1	0.529	26.467
	High-grade	7	68.8			3.74		
T stage	Low (T1 or T2)	74	165.9	< 0.001	< 0.001	1	2.501	31.709
	High (T3 or T4)	7	30.3			8.906		
Lymph node metastasis	Absent	79	NA	0.403	0.989	NA	NA	NA
	Present	2	NA					
Lymphovascular invasion	Absent	77	156.4	0.087	0.35	1	2.165	10.937
	Present	4	81			2.165		
Chemotherapy	None	37	176.4	0.054	0.423	1	2.490	23.194
	Platinum + Taxane	44	140.4			2.49		

† Cox proportional hazards model for multivariate analysis

ovarian mucinous histology. It is important to note that primary mucinous ovarian carcinoma is a rare entity, accounting for only 1–3% of all ovarian cancers. The majority of mucinous carcinomas in the ovary are known to be metastases from other organs [12, 13, 12], with the gastrointestinal tract being the most common primary site for metastatic ovarian carcinoma [13]. Wang et al. investigated CLDN18.2 expression in both primary and metastatic ovarian mucinous carcinomas. They reported CLDN18.2 expression in metastatic ovarian carcinomas originating from the upper gastrointestinal tract, but not in those of lower gastrointestinal origin [12]. Our study yielded similar results, with only a few cases of CLDN18.2 expression observed in metastatic ovarian carcinomas originating from the appendix (Table 2). The distinction between pseudomyxoma peritonei, ovarian carcinoma of appendix origin, and primary ovarian mucinous carcinoma remains a significant diagnostic challenge. Currently, no definitive biomarker has been identified to clearly differentiate between these entities [13, 12]. Given the high expression rate of CLDN18.2 in primary ovarian mucinous carcinomas and its limited expression in metastatic carcinomas of appendix origin, CLDN18.2 may serve as a valuable biomarker for distinguishing between primary ovarian mucinous carcinoma and metastatic mucinous carcinoma originating from the appendix.

Analysis of CLDN18.2 expression rates in primary tumors and their corresponding metastatic carcinomas revealed that expression levels were generally sustained, consistent with previous studies [12, 13]. CLDN18.2 expression was largely preserved during metastasis, although some variations in expression patterns were observed, particularly in gastric cancer. These findings underscore the dynamic nature of CLDN18.2 expression between primary and metastatic sites, especially in gastric malignancies. The variability in expression patterns between primary and metastatic lesions carries significant implications for targeted therapies and diagnostic approaches in managing these cancers. This study emphasizes the importance of evaluating CLDN18.2 expression in both primary and metastatic sites, as discordant cases, though relatively infrequent, may significantly impact treatment decisions and patient outcomes. The differences in expression patterns between primary and metastatic lesions could influence the efficacy of targeted therapies and the accuracy of diagnostic approaches.

While some studies have reported that CLDN18.2 expression decreases as cancer progresses, potentially contributing to tumor cell invasion and metastasis formation [13, 12], our study demonstrated that CLDN18.2 expression was largely preserved during metastatic dissemination. This apparent discrepancy highlights the complex role of CLDN18.2 in cancer progression and metastasis. Further research is warranted to elucidate the precise role of CLDN18.2 in the metastatic process and its interactions within the ovarian tumor microenvironment.

The prognostic significance of CLDN18.2 expression in cancer has been a subject of debate in recent studies. While Matsuoka et al. initially suggested that loss of CLDN18.2 expression might be a marker of poor prognosis in certain tumor types [13], subsequent investigations have failed to establish a clear correlation between CLDN18.2 expression and survival outcomes [12, 13, 12]. Our study, which evaluated the prognostic role of CLDN18.2 in a cohort of 102 patients with stage 4 cancer and ovarian metastasis, found no significant difference in overall survival (OS) based on CLDN18.2 expression. This finding supports the notion that CLDN18.2 is not an independent prognostic factor for overall survival, at least in this specific patient population.

CLDN18.2 expression has been observed in borderline ovarian mucinous tumors and benign ovarian mucinous tumors [13, 12]. Our study corroborated these findings, demonstrating CLDN18.2 expression in mucinous borderline tumor and mucinous carcinoma. This is particularly relevant given that mucinous ovarian carcinomas tend to occur in relatively younger patients and exhibit poor responses to platinum-based chemotherapy, leading to worse prognoses [12]. The observed CLDN18.2 overexpression in these tumors suggests that targeted therapies, such as zolbetuximab, could potentially improve survival rates in mucinous ovarian carcinoma.

Interestingly, CLDN18.2 overexpression has been reported in other mucinous carcinomas, including lung mucinous carcinoma and cervical gastric-type adenocarcinoma [4, 12]. Iwaya et al. proposed that aberrant CLDN18.2 expression in colitis-associated colorectal cancer might be linked to chronic inflammation and repeated mucosal damage, potentially associated with the reprogramming or conversion of colonic precursor cells into gastric epithelial cells [13]. These findings highlight the need for further research to elucidate the correlation between CLDN18.2 expression and mucinous-type adenocarcinoma across various organ systems.

CLDN18.2 expression should be considered alongside the type of primary tumor when planning treatment strategies. Moreover, the potential of certain chemotherapy regimens to delay metastasis warrants further investigation and may influence treatment prioritization. However, the precise role of CLDN18.2 in the tumor microenvironment and its influence on metastasis remains unclear. Exploring these mechanisms could provide crucial insights into preventing or delaying metastasis. Future research should focus on elucidating the complex interactions between CLDN18.2, the tumor microenvironment, and the metastatic process to enhance our understanding of cancer progression and improve patient outcomes.

While our study primarily focused on primary cancers of the cervix, breast, appendix, stomach, and colon, the large cohort size of matched cases and the extended timespan over which these samples were collected help to mitigate potential biases and enhance the robustness of our results. Nevertheless, we acknowledge that the relatively small sample sizes in certain subgroups—uterine cervical, appendiceal, and breast cancers—may constrain the statistical power of analyses within these specific groups. This limitation highlights the importance of validating our results in larger, independent cohorts to confirm their generalizability. Furthermore, our reliance on immunohistochemical analyses may not fully represent CLDN18.2 overexpression due to tumor heterogeneity or variations in the percentage of tumor cells within the overall tumor mass. Future studies incorporating complementary mRNA analysis could provide a more comprehensive understanding of CLDN18.2 gene expression.

The histopathological heterogeneity of ovarian mucinous tumors suggests that certain samples may have been differentially influenced by the coexistence of malignant, borderline, and benign components, potentially introducing variability in CLDN18.2 expression assessment. The inclusion of benign mucinous tumors as a comparative control would have provided valuable context for distinguishing CLDN18.2 expression patterns across different tumor types. Future studies should address this limitation by incorporating benign counterparts and conducting more detailed analyses across distinct histological regions within the same tumor to better delineate CLDN18.2 expression dynamics.

Additionally, the antibody used in this study detects both CLDN18.1 and CLDN18.2 isoforms due to their shared epitope homology. This lack of isoform specificity poses an interpretational challenge, particularly in tissues where both isoforms may coexist, such as the lung and gastrointestinal tract. While the tissue-specific expression patterns of CLDN18 isoforms suggest that most observed staining in our cohort likely represents CLDN18.2, the inability to definitively distinguish isoforms limits the precision of our findings. Further studies employing isoform-specific antibodies or integrating complementary mRNA analysis are warranted to provide a more comprehensive understanding and generate robust insights into CLDN18.2 gene expression.

Although our findings indicate that CLDN18.2 overexpression is not an independent prognostic factor, its potential survival benefit in metastatic settings makes it a promising candidate for targeted therapies such as zolbetuximab. This potential extends to both metastatic cancers and primary mucinous ovarian carcinoma, where CLDN18.2 demonstrates particular promise as a therapeutic target. The sustained expression of

CLDN18.2 in metastatic tumors suggests that targeting this protein could be considered a novel treatment option for patients with advanced disease. This is especially relevant for mucinous ovarian cancer, where current treatment options are limited and outcomes are often poor.

In conclusion, our study enhances the understanding of CLDN18.2 expression patterns in various cancer types and stages, providing a foundation for future research and potential therapeutic interventions. The preservation of CLDN18.2 expression during metastasis underscores its potential as a therapeutic target in both primary and metastatic ovarian mucinous cancers.

Authors' contributions

JHL and KHL designed this study. JYK and NIK drafted the manuscript. SSK and JYL performed experiments. JYK and JYL performed data analysis. NIK, BHJ and BWK collected clinical data. SSK, NIK, and JSL carried out a pathological examination. TMY and KSM assisted with the manuscript preparation and data analysis. KHL and TMY helped for funding acquisition. LJH and LKH supervised the study. All authors read and approved the final manuscript.

Funding

This work was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Minist (2022R1A2C1011889 and 2020R1A5A2031185 for KHL) and by Chonnam National University Hospital Biomedical Research Institute (HCRI21005 and HCRI23011 for KHL; HCRI21004 for YTM). There was no role of the funding bodies in the design of the study, in the collection, analysis, and interpretation of data or in writing the manuscript.

Data availability

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Review Board of Chonnam National University Hwasun Hospital (CNUHH-2024–108). Written informed consent to use clinical data & pathological samples was obtained from patients or their legal surrogates. All experiments were performed in accordance with the ethical standards of the institutional and/or national research committee and the Declaration of Helsinki 1964 and its later amendments.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Department of Pathology, Chonnam National University Hwasun Hospital and Medical School, 264 Seoyang-ro, Hwasun-eup, Hwasun-gun, Jeollanam-do 58128, Republic of Korea. [2]Division of Hematology-Oncology, Department of Internal Medicine, Chonnam National University Hwasun Hospital and Medical School, Hwasun, Republic of Korea. [3]Department of Otolaryngology-Head and Neck Surgery, Chonnam National University Hwasun Hospital and Medical School, Hwasun, Republic of Korea. [4]Department of Neurosurgery, Chonnam National University Hwasun Hospital and Medical School, Hwasun, Republic of Korea. [5]BioMedical Sciences Graduate Program (BMSGP), Chonnam National University, Hwasun, Republic of Korea.

Received: 3 January 2025 Accepted: 14 March 2025
Published online: 25 March 2025

References

1. Ding L, Lu Z, Lu Q, Chen YH. The claudin family of proteins in human malignancy: a clinical perspective. Cancer Manag Res. 2013;5:367–75.
2. Tsukita S, Tanaka H, Tamura A. The claudins: from tight junctions to biological systems. Trends Biochem Sci. 2019;44(2):141–52.
3. Hana C, Thaw Dar NN, Galo Venegas M, Vulfovich M. Claudins in cancer: a current and future therapeutic target. Int J Mol Sci. 2024;25(9):4634.
4. Kyuno D, Takasawa A, Takasawa K, Ono Y, Aoyama T, Magara K, Nakamori Y, Takemasa I, Osanai M. Claudin-18.2 as a therapeutic target in cancers: cumulative findings from basic research and clinical trials. Tissue Barriers. 2022;10(1):1967080.
5. Nakayama I, Qi C, Chen Y, Nakamura Y, Shen L, Shitara K. Claudin 18.2 as a novel therapeutic target. Nat Rev Clin Oncol. 2024;21(5):354–69.
6. van Laarhoven HWM, Derks S. Claudin-18.2 targeting by zolbetuximab: results of SPOTLIGHT in perspective. Lancet. 2023;401(10389):1630–1.
7. Niclauss N, Gütgemann I, Dohmen J, Kalff JC, Lingohr P. Novel biomarkers of gastric adenocarcinoma: current research and future perspectives. Cancers (Basel). 2021;13(22):5660.
8. Derks S, van Laarhoven HWM. SPOTlight on GLOW. Cell Rep Med. 2023;4(10):101233.
9. Amin MB, Edge SB, Greene FL, Byrd DR, Brookland RK, Washington MK, Gershenwald JE, Compton CC, Hess KR, Sullivan DC. AJCC cancer staging manual. Chicago: Springer International Publishing; 2018.
10. WHO Classification of Tumours Editorial Board. Female genital tumours: WHO classification of tumours. Lyon: International Agency for Research on Cancer; 2020.
11. WHO Classification of Tumours Editorial Board. Digestive system tumours: WHO classification of tumours. Lyon: International Agency for Research on Cancer; 2019.
12. Shah MA, Shitara K, Ajani JA, Bang YJ, Enzinger P, Ilson D, Lordick F, Van Cutsem E, Gallego Plazas J, Huang J, et al. Zolbetuximab plus CAPOX in CLDN18.2-positive gastric or gastroesophageal junction adenocarcinoma: the randomized, phase 3 GLOW trial. Nat Med. 2023;29(8):2133–41.
13. Shitara K, Lordick F, Bang YJ, Enzinger P, Ilson D, Shah MA, Van Cutsem E, Xu RH, Aprile G, Xu J, et al. Zolbetuximab plus mFOLFOX6 in patients with CLDN18.2-positive, HER2-negative, untreated, locally advanced unresectable or metastatic gastric or gastro-oesophageal junction adenocarcinoma (SPOTLIGHT): a multicentre, randomised, double-blind, phase 3 trial. Lancet. 2023;401(10389):1655–68.
14. Kim HD, Choi E, Shin J, Lee IS, Ko CS, Ryu MH, Park YS. Clinicopathologic features and prognostic value of claudin 18.2 overexpression in patients with resectable gastric cancer. Sci Rep. 2023;13(1):20047.
15. Kubota Y, Kawazoe A, Mishima S, Nakamura Y, Kotani D, Kuboki Y, Bando H, Kojima T, Doi T, Yoshino T, et al. Comprehensive clinical and molecular characterization of claudin 18.2 expression in advanced gastric or gastroesophageal junction cancer. ESMO Open. 2023;8(1): 100762.
16. Qi C, Gong J, Li J, Liu D, Qin Y, Ge S, Zhang M, Peng Z, Zhou J, Cao Y, et al. Claudin18.2-specific CAR T cells in gastrointestinal cancers: phase 1 trial interim results. Nat Med. 2022;28(6):1189–98.
17. Wang X, Zhang CS, Dong XY, Hu Y, Duan BJ, Bai J, Wu YY, Fan L, Liao XH, Kang Y, et al. Claudin 18.2 is a potential therapeutic target for zolbetuximab in pancreatic ductal adenocarcinoma. World J Gastrointest Oncol. 2022;14(7):1252–64.
18. Cao W, Xing H, Li Y, Tian W, Song Y, Jiang Z, Yu J. Claudin18.2 is a novel molecular biomarker for tumor-targeted immunotherapy. Biomark Res. 2022;10(1):38.
19. Chen J, Xu Z, Hu C, Zhang S, Zi M, Yuan L, Cheng X. Targeting CLDN18.2 in cancers of the gastrointestinal tract: New drugs and new indications. Front. Front Oncol. 2023;13:1132319.
20. Yan P, Dong Y, Zhang F, Zhen T, Liang J, Shi H, Han A. Claudin18.2 expression and its clinicopathological feature in adenocarcinoma from various parts. J Clin Pathol. 2024:jcp-2023-209268. https://doi.org/10.1136/jcp-2023-209268. Online ahead of print.
21. Arnold A, Daum S, von Winterfeld M, Berg E, Hummel M, Rau B, Stein U, Treese C. Prognostic impact of Claudin 18.2 in gastric and esophageal adenocarcinomas. Clin Transl Oncol. 2020;22(12):2357–63.

22. Rohde C, Yamaguchi R, Mukhina S, Sahin U, Itoh K, Türeci Ö. Comparison of claudin 18.2 expression in primary tumors and lymph node metastases in Japanese patients with gastric adenocarcinoma. Jpn J Clin Oncol. 2019;49(9):870–6.
23. Wagner P, Gass P, Pöschke P, Eckstein M, Gloßner L, Hartmann A, Beckmann MW, Fasching PA, Ruebner M, Emons J, et al. Spatial expression of claudin 18.2 in matched primaries and metastases of tubo-ovarian carcinoma of all subtypes. Virchows Arch. 2024;485(1):63–74.
24. Lheureux S, Gourley C, Vergote I, Oza AM. Epithelial ovarian cancer. Lancet. 2019;393(10177):1240–53.
25. Perren TJ. Mucinous epithelial ovarian carcinoma. Ann Oncol. 2016;27(Suppl 1):i53–7.
26. Morice P, Gouy S, Leary A. Mucinous ovarian carcinoma. N Engl J Med. 2019;380(13):1256–66.
27. Park CK, Kim HS. Clinicopathological characteristics of ovarian metastasis from colorectal and pancreatobiliary carcinomas mimicking primary ovarian mucinous tumor. Anticancer Res. 2018;38(9):5465–73.
28. Wang F, Yang Y, Du X, Zhu X, Hu Y, Lu C, Sui L, Zhao H, Song K, Yao Q. Claudin18.2 as a potential therapeutic target for primary ovarian mucinous carcinomas and metastatic ovarian mucinous carcinomas from upper gastrointestinal primary tumours. BMC Cancer. 2023;23(1):44.
29. Perez Montiel D, Arispe Angulo K, Cantú-de León D, Bornstein Quevedo L, Chanona Vilchis J, Herrera Montalvo L. The value of SATB2 in the differential diagnosis of intestinal-type mucinous tumors of the ovary: primary vs metastatic. Ann Diagn Pathol. 2015;19(4):249–52.
30. Dundr P, Singh N, Nožičková B, Němejcová K, Bártů M, Stružinská I. Primary mucinous ovarian tumors vs. ovarian metastases from gastrointestinal tract, pancreas and biliary tree: a review of current problematics. Diagn Pathol. 2021;16(1):20.
31. Jun KH, Kim JH, Jung JH, Choi HJ, Chin HM. Expression of claudin-7 and loss of claudin-18 correlate with poor prognosis in gastric cancer. Int J Surg. 2014;12(2):156–62.
32. Oshima T, Shan J, Okugawa T, Chen X, Hori K, Tomita T, Fukui H, Watari J, Miwa H. Down-regulation of claudin-18 is associated with the proliferative and invasive potential of gastric cancer at the invasive front. PLoS ONE. 2013;8(9): e74757.
33. Matsuoka T, Mitomi H, Fukui N, Kanazawa H, Saito T, Hayashi T, Yao T. Cluster analysis of claudin-1 and -4, E-cadherin, and β-catenin expression in colorectal cancers. J Surg Oncol. 2011;103(7):674–86.
34. Hong JY, An JY, Lee J, Park SH, Park JO, Park YS, Lim HY, Kim KM, Kang WK, Kim ST. Claudin 18.2 expression in various tumor types and its role as a potential target in advanced gastric cancer. Transl Cancer Res. 2020;9(5):3367–74.
35. Halimi SA, Maeda D, Shinozaki-Ushiku A, Koso T, Matsusaka K, Tanaka M, Arimoto T, Oda K, Kawana K, Yano T, et al. Claudin-18 overexpression in intestinal-type mucinous borderline tumour of the ovary. Histopathology. 2013;63(4):534–44.
36. Halimi SA, Maeda D, Ushiku-Shinozaki A, Goto A, Oda K, Osuga Y, Fujii T, Ushiku T, Fukayama M. Comprehensive immunohistochemical analysis of the gastrointestinal and Müllerian phenotypes of 139 ovarian mucinous cystadenomas. Hum Pathol. 2021;109:21–30.
37. Iwaya M, Hayashi H, Nakajima T, Matsuda K, Kinugawa Y, Tobe Y, Tateishi Y, Iwaya Y, Uehara T, Ota H. Colitis-associated colorectal adenocarcinomas frequently express claudin 18 isoform 2: implications for claudin 18.2 monoclonal antibody therapy. Histopathology. 2021;79(2):227–37.

Publisher's Note