Clinical Significance of CLDN18.2 Expression in Diffuse-Type Metastatic Gastric Cancer

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Abstract

Background Isoform 2 of the tight junction protein claudin-18 (CLDN18.2) is a potential target of gastric cancer treatment. A treatment targeting CLDN18.2 has shown promising results in gastric cancer. We investigated the clinical significance of CLDN18.2 and other cell-adherens junction molecules (Rho GTPase-activating protein [RhoGAP] and E-cadherin) in metastatic diffuse-type gastric cancer (mDGC).

Methods We evaluated CLDN18.2, RhoGAP, and E-cadherin expression by performing two-plex immunofluorescence and quantitative data analysis of H-scores of 77 consecutive mDGC patients who received first-line, platinum-based chemotherapy between March 2015 and February 2017.

Results CLDN18.2 and E-cadherin expression was significantly reduced in patients with peritoneal metastasis (PM) compared with those without PM at the time of diagnosis (P=0.010 and 0.013, respectively), while it was significantly higher in patients who never exhibited PM from diagnosis to death than in those who did (p=0.001 and 0.003, respectively). Meanwhile, CLDN18.2 and E-cadherin were expressed at significantly higher levels in patients with bone metastasis than in those without it (p=0.010 and 0.001, respectively). We identified a positive correlation between the expression of CLDN18.2 and E-cadherin ($p < 0.001$), RhoGAP and CLDN18.2 (p=0.004), and RhoGAP and E-cadherin (p=0.001). CDLN18.2, RhoGAP, and E-cadherin expression was not associated with chemotherapy response and survival.

Conclusions CLDN18.2 expression was reduced in PM but significantly intact in bone metastasis. Furthermore, a positive correlation was identified between the expression of CLDN18.2 and other adherens junction molecules, which has clinical implications for mDGC and PM pathogenesis.

Introduction

Gastric cancer (GC) is the fourth most common cancer in the world and the second leading cause of cancer-related death [1]. It has been categorized into two main histological subtypes based on Lauren's criteria: intestinal and diffuse [2]. Diffuse-type gastric cancer (DGC) is associated with worse prognosis, occurrence at an earlier age, and highest recurrence frequency [3]. DGCs are poorly differentiated histologically; they are characterized by a lack of intercellular adhesion molecules and disrupted tight junction molecules, and often have poorly cohesive and scattered signet-ring cell morphology, which is predisposed to invasion growth patterns [4]. DGC is associated with peritoneal metastasis (PM) more frequently than intestinal-type GC [5]. PM is the most frequent metastatic pattern of GC, but the mechanisms underlying peritoneal dissemination are yet to be elucidated, although Paget's 'seed and soil' hypothesis has been considered as the fundamental theory of metastasis [6]. According to this hypothesis, detachment of cancer cells from the primary tumor is the first step in peritoneal dissemination; thus, cancer cell detachment is considered important in PM [7]. A previous study suggested that the loss of cell-cell adhesion systems results in the dissemination of single carcinoma cells from the primary tumor sites and triggers remodeling of the actin cytoskeleton, leading to the development of a mesenchymal phenotype and dispersal of carcinoma cells [8]. The importance of the

loss of cell–cell adhesion molecules such as E-cadherin in the context of DGC initiation and PM formation has been evaluated in several studies [9].

Claudin-18 (CLDN18) is a member of the claudin family and is a component of tight junctions that regulate paracellular barrier functions. Several studies have suggested that DGC is associated with an inter-chromosomal translocation between CLDN18 and *ARHGAP* (gene encoding Rho GTPase-activating protein [RhoGAP], which contributes to the organization of the actin and microtubule cytoskeletons), which results in the generation of a RhoGAP domain-containing fusion protein in which the function of CLDN18 and RhoGAP is likely impaired [10–14]. A previous in vitro study reported that CLDN18–ARHGAP26 fusion-positive cell lines showed impaired barrier properties, reduced cell–cell adhesion, and augmented invasiveness [15]. Also, other previous studies suggested that CLDN18-ARHGAP26/6 or the RhoGAP domain-containing fusion protein was associated with poor prognosis of DGC [11, 13].

The expression of the isoform 2 of CLDN18 (CLDN18.2) has been shown to be limited to differentiated epithelial cells in the gastric mucosa and primary gastric malignancies, emphasizing its potential as a candidate for targeted therapy [16]. Recently, a treatment strategy targeting CLDN18.2 has shown promising results in inoperable or recurrent GC patients [17, 18].

In this study, we aimed to investigate the clinical significance of CLDN18.2 and other adherens junction molecules (RhoGAP and E-cadherin) in metastatic DGC (mDGC).

Methods

Study population and design

A total of 77 patients with mDGC who were treated with first-line, platinum-based chemotherapy between March 2015 and February 2017 in Seoul St. Mary's hospital, the Catholic University of Korea were included in this study. We performed gastric endoscopic biopsy of the primary gastric tumor lesion. Computed tomography (CT) and bone scan were performed for staging. If needed, additional imaging scans such as magnetic resonance imaging and positron emission tomography/CT were performed. Patients were subsequently evaluated for their response to chemotherapy after 6 ± 2 weeks based on radiological imaging results. Radiological changes were evaluated using the Response Evaluation Criteria in Solid Tumors version 1.1 [19]. The tumor location was classified as upper/middle/lower third. An immunohistochemical score of 3+ or 2+ was considered for determining human epidermal growth factor receptor 2 positivity by fluorescence in situ hybridization analysis. Diagnostic laparoscopy for PM was performed in patients for whom a diagnosis of PM was unclear in the radiological examination. If the patient showed definite PM on CT or other imaging modalities, diagnostic laparoscopy was not performed.

Ethical statement

This study was approved by the Institutional Review Board of Seoul St. Mary's Hospital, and written informed consent was obtained from all patients.

Assessment of CLDN18.2, RhoGAP, and E-cadherin expression by two-plex immunofluorescence

Sections of GC specimens (4 μm) were cut from formalin-fixed, paraffin-embedded blocks. Samples were heated for at least 1 h in a dry oven at 60°C, dewaxed using xylene, dehydrated by sequential incubation with 100%, 95%, and 70% ethanol, followed by hydrogen peroxide treatment. Antigens were retrieved by microwave treatment for 15 min in citrate buffer (pH 6.0). Slides were washed with 1x Tris-buffered saline with Tween 20® two times, and blocking was performed by using an antibody diluent (#ARD1001EA, PerkinElmer, Waltham, MA) for 10 min. Samples were incubated with primary antibodies against CLDN18.2 (#700178, Invitrogen, Carlsbad, CA; dilution 1:500), RhoGAP (#ab32328, Abcam, Cambridge, UK; dilution 1:1000), and E-cadherin (#ab76055, Abcam; dilution 1:400) for 30 min in a humidified chamber at room temperature, followed by detection using the Polymer HRP Ms+Rb (ARH1001EA, PerkinElmer) for 10 min. Visualization of CLDN18.2, RhoGAP, and E-cadherin was accomplished by incubation with Opal 690 TSA Plus (dilution 1:150) for 10 min, after which the samples immobilized on the slides were immersed in citrate buffer (pH 6.0) and heated by microwave treatment. The nuclei were subsequently visualized using detecting nuclear spectral elements (DAPI), and the sections were mounted using HIGHDEF® immunohistochemical fluoromount (#ADI-950-260-0025, Enzo).

Image acquisition and quantitative data analysis

Slides were scanned using the PerkinElmer Vectra 3.0 Automated Quantitative Pathology Imaging System (PerkinElmer) and images were analyzed using the inForm software and TIBCO Spotfire (PerkinElmer). To acquire reliable unmixed images, representative slides exposed to each emission spectrum and unstained tissue slides were used. Each of the individually stained sections (E-cadherin, RhoGAP, and claudin-18; Opal690) were used to establish the spectral library of fluorophores required for multispectral analysis. This spectral library served as a reference for target quantitation as the intensity of each fluorescent target was extracted from the multispectral data by linear unmixing. Each cell was identified by DAPI. The total number of CLDN18.2-, RhoGAP-, and E-cadherin-positive cells was considered as the total number of cell infiltrations in the tissue. The fluorescence intensity of CLDN18.2, RhoGAP, and E-cadherin in the tissue samples was determined based on H-score. The intensity was determined based on tumor cells showing strong (3+), intermediate (2+), weak (1+), or no (0) membranous staining of CLDN18.2, RhoGAP, and E-cadherin. The H-score of each tissue sample was calculated using the following formula: H-score = [(3 × the percentage of strongly stained) + (2 × the percentage of moderately stained) + (the percentage of weakly stained)]. H-scores ranged from 0 to 300.

Statistical analysis

The correlation between the H-score of CLDN18.2, RhoGAP, and E-cadherin, and the clinicopathological factors and radiological responses were analyzed using the independent-sample *t*-test. The correlation between H-scores among each marker was assessed by performing Pearson's correlation analysis.

Overall survival (OS) and progression-free survival (PFS) were calculated from the start date of first-line palliative chemotherapy until the date of death, and disease progression or death, respectively. For survival analyses, data from living patients or those with no disease progression were censored from the last follow-up date. OS and PFS were analyzed by the Kaplan–Meier method, and the log-rank test and multivariate analysis. All analyses were performed using the SPSS software (version 24; IBM Corp., Armonk, NY, USA), and a two-sided p-value of < 0.05 was considered statistically significant.

Results

Baseline patient characteristics and CLDN18.2, RhoGAP, and E-cadherin expression

A total of 77 patients were included in the present study. Patient characteristics are listed in Table 1. The median age of the patients was 52 years (range, 27–82 years). The patient cohort comprised 35 (45.5%) men and 42 (54.5%) women. PM was observed in 50 (64.9%) patients. Distant lymph node (LN), liver, and bone metastases were observed in 34 (44.2%), 9 (11.7%), and 12 (15.6%) patients, respectively.

Table 1
Baseline characteristics (N = 77)

Characteristic	Data
Age (years)	52 (27–82)
< 45	23 (29.9)
≥ 45	54 (70.1)
Sex	
Female	42 (54.5)
Male	35 (45.5)
Tumor location	
Upper third	14 (18.2)
Middle third	36 (46.8)
Lower third	27 (35.1)
cT stage	
1	2 (2.6)
2	6 (7.8)
3	19 (24.7)
4	50 (64.9)
cN stage	
0	14 (18.2)
1	9 (11.7)
2	30 (39.0)
3	24 (31.2)
HER2	
Negative	57 (74.0)
Positive	10 (13.0)
Unknown	10 (13.0)

Data are expressed as median (range) or number (%).

cT, stage clinical T stage; cN stage, clinical N stage; HER2, human epidermal growth factor receptor 2; LN lymph node.

Characteristic	Data
Peritoneal metastasis	
No	27 (35.1)
Yes	50 (64.9)
Distant LN metastasis	
No	43 (55.8)
Yes	34 (44.2)
Liver metastasis	
No	68 (88.3)
Yes	9 (11.7)
Bone metastasis	
No	65 (84.4)
Yes	12 (15.6)
Data are expressed as median (range) or number (%).	
cT, stage clinical T stage; cN stage, clinical N stage; HER2, human epidermal growth factor receptor 2; LN lymph node.	

Evaluation of CLDN18.2, RhoGAP, and E-cadherin expression

CLDN18.2, RhoGAP, and E-cadherin expression was evaluated using whole tissue sections. Fig. 1 shows the expression pattern of CLDN18.2, RhoGAP, and E-cadherin. The mean H-score of CLDN18.2, RhoGAP, and E-cadherin was 45 (min–max, 0–170), 17 (0–70), and 54 (0–150), respectively. The distribution of these markers are summarized in Fig. 2.

Correlation between clinicopathological factors and H-scores of CLDN18.2, RhoGAP, and E-cadherin

We investigated the correlation between cell-adherens junction protein expression and clinicopathological factors (Table 2 and Fig. 3). CLDN18.2 expression was significantly reduced in patients with PM compared to those without PM (Mean H-scores, 36.98 vs. 60.67, p=0.010). In contrast, CLDN18.2 was expressed at significantly higher levels in patients with bone metastasis than in those without it (78.19 vs. 39.22, p=0.010). Additionally, CLDN18.2 expression was lower in patients who were young (< 45 years old) and in those with liver metastases. There were no other significant correlations between clinicopathological factors and CLDN18.2 expression. E-cadherin expression was lower in patients with PM than in those without PM (46.74 vs. 68.63, p=0.013). In contrast, E-cadherin expression was higher in patients with bone metastases (89.27 vs. 47.85, p=0.001). There were no other significant correlations between clinicopathological factors and E-cadherin expression. RhoGAP expression was not correlated with any of the clinicopathological factors.

At the time of progression after receiving first-line chemotherapy, among the 27 patients who did not exhibit PM initially, 12 patients showed new PM while 15 patients still did not. Expression of CLDN18.2 and E-cadherin was significantly higher in these 15 patients than in patients with PM (CLDN18.2, 73.35 vs. 39.05, p=0.002; E-cadherin, 81.04 vs. 48.36, p=0.002). Among the 15 patients who did not show PM at the time of progression after receiving first-line chemotherapy, 5 patients did not show PM until death. Expression of CLDN18.2 and E-cadherin was significantly higher in these 5 patients than that in the 72 patients who developed PM (CLDN18.2, 99.89 vs. 41.50, p=0.001; E-cadherin, 101.31 vs. 51.04, p=0.003) (Fig. 3).

Table 2
Correlation between cell-adherens junction proteins and clinicopathologic factors (N = 77)

CLDN18.2			RhoGAP			E-cadherin		
mean ± SD	**t**	**p**	**mean ± SD**	**t**	**p**	**mean ± SD**	**t**	**p**
Age								
< 45 (n = 23)	32.75 ± 27.11	-2.222	0.030*	17.67 ± 12.43	0.056	0.956	46.56 ± 36.55	-1.206	0.232
≥ 45 (n = 54)	50.63 ± 42.04	17.48 ± 14.43	57.60 ± 36.89						
Peritoneal metastasis									
No (n = 27)	60.67 ± 44.19	2.648	0.010*	18.48 ± 14.48	0.436	0.664	68.63 ± 40.40	2.532	0.013*
Yes (n = 50)	36.98 ± 33.32	17.03 ± 13.51	46.74 ± 32.85						
Liver metastasis									
No (n = 68)	47.91 ± 40.16	2.784	0.012*	17.65 ± 11.54	0.191	0.849	55.74 ± 37.60	0.937	0.352
Yes (n = 9)	25.52 ± 19.19	16.71 ± 37.60	43.46 ± 30.76						
Bone metastasis									
No (n = 65)	39.22 ± 35.24	-2.975	0.010*	17.62 ± 14.23	0.128	0.899	47.85 ± 33.57	-3.89	0.001*
Yes (n = 12)	78.19 ± 42.78	17.07 ± 11.54	89.27 ± 35.63						
CLDN 18.2, ; RhoGAP, ; SD, standard deviation.									
*Statistically significant.									

Correlation between CLDN18.2, RhoGAP, and E-cadherin expression

We investigated the correlation between CLDN18.2, RhoGAP, and E-cadherin expression (Fig. 4). CLDN18.2 expression was positively correlated with that of E-cadherin (r=0.765, p<0.001). RhoGAP expression was positively correlated with that of CLDN18.2 (r=0.325, p=0.004) and E-cadherin (r=0.373, p=0.001).

Survival and chemotherapy response based on CLDN18.2, RhoGAP, and E-cadherin expression

We investigated the tumor response after first-line chemotherapy treatment based on CLDN18.2, RhoGAP, and E-cadherin expression (Table 3). Of the 77 patients, 54 patients had a measurable lesion. CLDN18.2 levels were not significantly different between the objective response (complete response [CR]/partial response [PR]) and other (stable disease [SD]/progressive disease [PD]) groups (51.75 vs. 32.01, p=0.052). RhoGAP and E-cadherin expression levels were also not statistically different between the CR/PR and SD/PD groups. CDLN18.2, RhoGAP, and E-cadherin levels were not different between the disease control (CR/PR/SD) and PD groups. OS and PFS in all patients were investigated based on CLDN18.2, RhoGAP, and E-cadherin expression. CDLN18.2, RhoGAP, and E-cadherin positivity was determined based on the median value (Supplementary Fig. 1). CDLN18.2, RhoGAP, and E-cadherin positivity was not associated with OS and PFS according to univariate and multivariate analyses.

Table 3
First-line chemotherapy best response based on CLDN18.2, RhoGAP and E-cadherin expression (N = 77)

CLDN18.2			**RhoGAP**			**E-cadherin**		
mean ± SD	**t**	**p**	**mean ± SD**	**t**	**p**	**mean ± SD**	**t**	**p**
Chemotherapy response								
CR/PR (n = 25)	51.75 ± 43.28	1.987	0.052	22.08 ± 17.63	1.546	0.131	60.37 ± 37.83	1.68	0.099
SD/PD (n = 29)	32.01 ± 29.24	15.95 ± 9.75	43.98 ± 33.87					
Peritoneal metastasis								
CR/PR/SD (n = 43)	43.53 ± 38.6	0.925	0.359	19.86 ± 15.27	1.602	0.119	55.16 ± 36.19	1.451	0.153
PD (n = 11)	31.82 ± 32.17	14.06 ± 7.67	37.52 ± 35.14					

CLDN 18.2, ; RhoGAP, ; SD, standard deviation; CR, complete response; PR, complete/partial response; SD, stable disease; PD, progressive disease.

Discussion

The present study aimed to evaluate the clinical significance of CLDN18.2 expression in mDGC. To the best of our knowledge, this is the first study to investigate the clinical significance of CLDN18.2 expression by immunofluorescence in mDGCs.

We investigated the association between the expression of adherens junction molecules and PM. Several previous studies have suggested that the loss of adherens junction structure stability is associated with PM. Yonemura et al. [9] have suggested that reduced expression of E-cadherin and high expression of S100A4 promote PM, serosal involvement, and infiltrative tumor growth. Togano et al. [20] have suggested that loss of E-cadherin expression is a critical step for the PM of GC with sub-serosal invasion. To date, little is known about the clinical impact of CLDN18.2 expression in metastatic GC. Previous studies have suggested that loss of CLDN18.2 expression is associated with an increased proliferative and invasive potential of GC [21, 22]. We found that CLDN18.2 and E-cadherin expression was decreased in patients with PM compared to those without PM. Interestingly, CLDN18.2 and E-cadherin expression was significantly higher in patients who never experienced PM until death than in patients who developed PM. Our result suggests that adherens junction instability may be involved in the progression and formation of PM during the course of the disease.

Remarkably, CLDN18.2 and E-cadherin expression was significantly higher in patients with bone metastases in the present study. Actually, bone metastasis and PM in our cohort showed a nearly exclusive pattern (PM rates: 17% [2/12] in patients with bone metastasis and 74% [48/65] in patients without bone metastasis). Furthermore, among the 5 patients who never experienced PM until death, 3 patients had initial bone metastasis. These results are in line with those reported in previous studies which showed that bone metastasis is not frequently synchronous with PM [23]. These results suggest that the mechanism of organotropic metastasis is clearly different. Our results suggest that loss of adherens junction integrity, which is generally accepted as the first step in PM formation, is more important in PM than in metastases to other sites such as bone, which occurs mostly through hematogenous spreading [24].

We found a positive correlation between CLDN18.2, RhoGAP, and E-cadherin expression. Several studies have proposed expression-related interactions between different adherens junction proteins. Lu et al. [25] investigated the function of claudin-7 with respect to the regulation of cell proliferation and maintenance of epithelial-cell attachment via engagement of integrin β1. Wu et al. [26] suggested that EpCAM modulates adhesion and tight junction function by regulating the intracellular localization and degradation of selected claudins. To date, the precise mechanism of interaction between adherens junction proteins is not well understood. A previous study had suggested that intercellular junctions not only come apart but also undergo regulatory changes including a phenomenon known as a cadherin switch, in which epithelial cells lose E-cadherin expression and begin to express N-cadherin during epithelial-mesenchymal transition [27]. Our findings cannot explain the mechanism of interaction between CDLN18.2, RhoGAP, and E-cadherin. The positive correlation between the expression of these adherens junction molecules may result in their ability to disrupt cellular cohesiveness synergistically. Further studies investigating these mechanisms are needed.

We found no difference in chemotherapy response and survival with respect to the expression of adherens junction molecules. A previous study had suggested that cell-adherens junction molecules play an important role in the epithelial-mesenchymal transition and influence the chemosensitivity of cancer cells [28]. Skalova et al. [29] proposed that claudin-1 and claudin-3 play a role in the response to chemotherapy in breast cancer. Yang et al. [13] have suggested that patients with in-frame fusion genes containing the RhoGAP domain represent the aggressive subset of DGCs. Wang et al. [30] have suggested that loss of E-cadherin is associated with poor prognosis. However, there is no clear consensus regarding the chemosensitivity and prognosis of cell-adherens junction molecules in mDGCs. In addition, our study could not fully investigate the chemotherapy response due to the small subset size (54/77, 70%) because all the patients did not have a measurable lesion, which is a characteristic feature of metastatic GC. Furthermore, the present study is retrospective in nature and is therefore not appropriate for investigating survival. Further studies are needed.

Our study has several limitations. First, the association between cell-adherens junction molecules was not confirmed by other methods. Recent studies have investigated these markers using several methods such as whole genome sequencing and RNA sequencing [11, 13, 14]. A previous study had found that the fusion of *CLDN18.2* and *ARHGAP26*, which includes the RhoGAP domain, was observed frequently in mDGC [12]. However, immunostaining is a practical method for expression analysis. A recent clinical trial investigating an anti-CLDN18.2 antibody for treating GC used immunohistochemistry as a predictive marker; thus, we believe our study has clinical implications. Furthermore, digital pathology has several advantages with respect to accuracy. As there is currently no optimal cut-off or standard for evaluating these markers, our results can provide valuable clinical information to clinicians evaluating specimens by immunostaining. Additionally, our cohort included a limited number of patients. Therefore, the results should be interpreted with caution. An extensive study with a larger sample size is needed to confirm our findings. Finally, our study is retrospective and a prospective study is still required.

Overall, we evaluated the clinical significance of cell-adherens junction molecules including CLDN18.2, which are being investigated as targets in GC treatment. We found that CLDN18.2 expression was significantly reduced in patients with PM but intact in those with bone metastasis, which may be associated with the first step of the “seed and soil” model, rather than with hematogenous metastasis. We also found a positive correlation between the expression of CLDN18.2 and other adherens junction molecules, which has clinical implications for DGC and PM pathogenesis. Further studies are needed to confirm our results.

Abbreviations

CLDN18.2
Isoform 2 of claudin-18; RhoGAP:Rho GTPase-activating protein; mDGC:metastatic diffuse-type gastric cancer; PM:peritoneal metastasis; GC:Gastric cancer; DGC:Diffuse-type gastric cancer; CLDN18:Claudin-18; CLDN18.2:isoform 2 of CLDN18 ; CT:Computed tomography; OS:Overall survival; PFS:progression-free

survival; LN:lymph node; CR:complete response; PR:partial response; SD:stable disease; PD:progressive disease

Declarations

Ethical statement

This study was approved by the Institutional Review Board of Seoul St. Mary's Hospital, and written informed consent was obtained from all patients.

Ethics approval and consent to participate

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

Conflict of interest relevant to this article was not reported.

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Authors' contributions

In-Ho Kim generated conception of the work; Seo Ree Kim, Kabsoo Shin, Jae Myung Park, Han Hong Lee, Kyo Yong Song, Sung Hak Lee, Bohyun Kim, Sang-Yeob Kim, Seo Junyoung, Jeong-Oh Kim, Sang-Young Roh, In-Ho Kim got acquisition, analysis, and interpretation of the data; In-Ho Kim, Sang-Yeob Kim, Seoree Kim have drafted the work; and In-Ho Kim, Seo Junyoung, Seoree Kim substantially revised it. All authors reviewed the manuscript.

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Figures

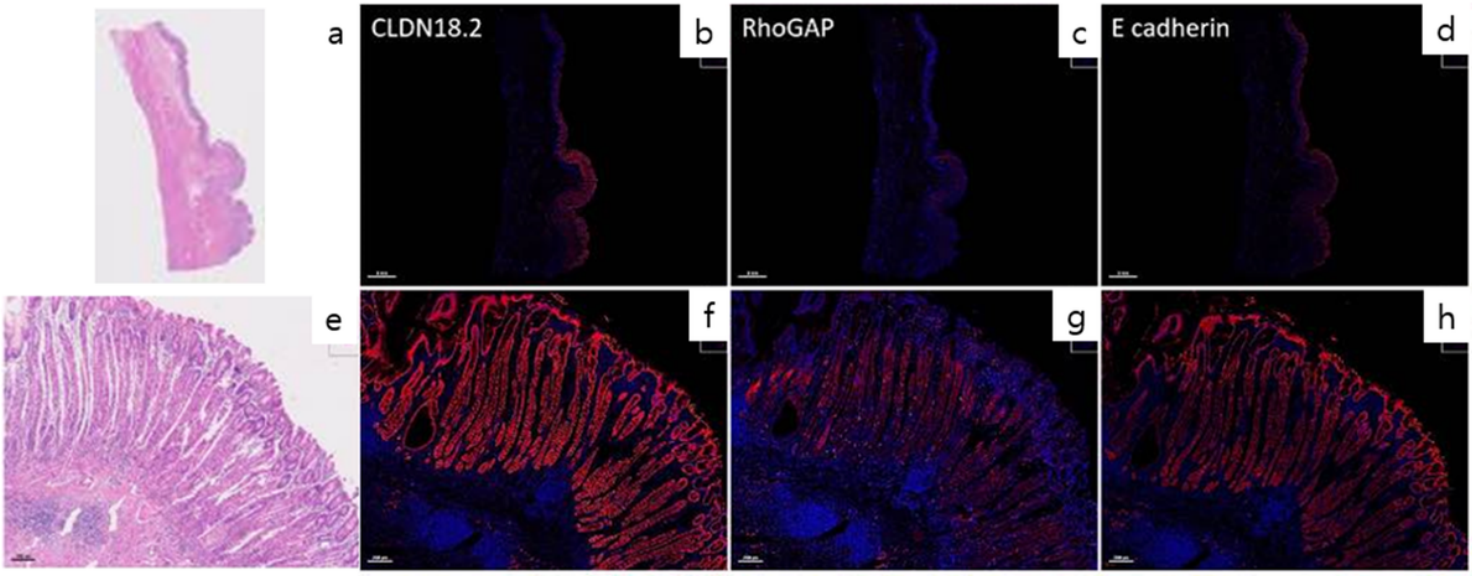

Figure 1

The expression of CLDN18.2, RhoGAP and E-cadherin using two-plex immunofluorescence. a, e) H&E; b, f) 3+/strong positive of CLDN18.2; c, g) 1+/weak positive of RhoGAP; d, h) 2+/intermediate positive of E-cadherin. CLDN18.2, isoform of claudin-18; RhoGAP, Rho GTPase-activating protein.

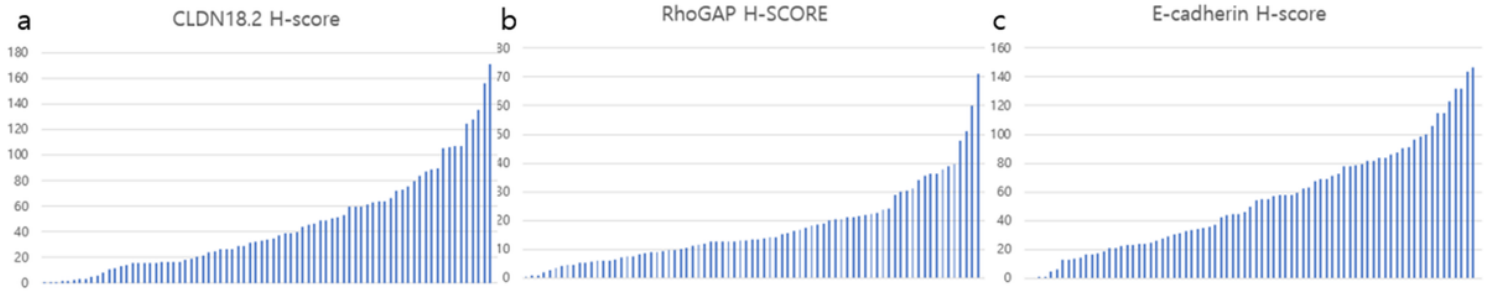

Figure 2

The H-score distribution of CLDN18.2 (a), RhoGAP (b) and E-cadherin (c). The mean H-score of CLDN18.2, RhoGAP, and E-cadherin was 45 (min–max, 0 –170), 17 (0–70), and 54 (0–150), respectively. CLDN18.2, isoform of claudin-18; RhoGAP, Rho GTPase-activating protein.

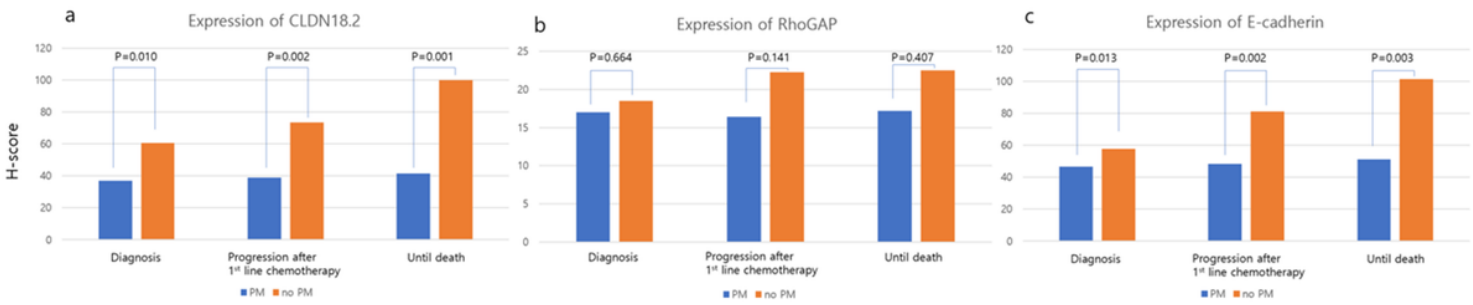

Figure 3

The expression of CLDN18.2 (a), RhoGAP (b) and E-cadherin (c) according to PM presence during disease course. Patients who never exhibited PM until death showed significantly higher expression of CLDN18.2 and E-cadherin. CLDN18.2, isoform of claudin-18; RhoGAP, Rho GTPase-activating protein; PM, peritoneal metastasis.

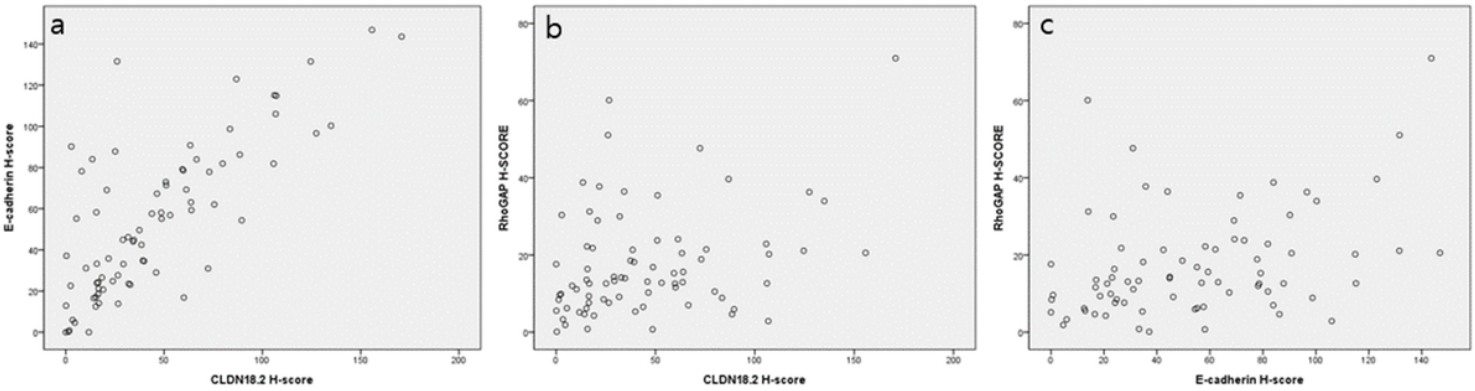

Figure 4

The correlation between CLDN18.2, RhoGAP and E-cadherin. The expression of CLDN18.2 had a positive association with that of E-cadherin (r=0.765, p < 0.001) (a). RhoGAP expression was positively associated with CLDN18.2 (r=0.325, p=0.004) (b) and E-cadherin (R=0.373, p=0.001) (c) expression. CLDN18.2, isoform of claudin-18; RhoGAP, Rho GTPase-activating protein.

Supplementary Files

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