Claudin-4 expression is associated with tumor invasion, MMP-2 and MMP-9 expression in gastric cancer

TSANN-LONG HWANG[1], LI-YU LEE[2], CHEE-CHAN WANG[3], YING LIANG[4], SHU-FANG HUANG[1] and CHI-MING WU[3]

Departments of [1]Surgery, and [2]Pathology, Chang Gung Memorial Hospital; [3]Department of Cosmetic Science, Vanung University; [4]Graduate Institute of Basic Medical Science, Chang Gung University, Tao-Yuan, Taiwan, R.O.C.

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Abstract. Claudin-4 is a member of the claudin family, a large family of transmembrane proteins that are essential in the formation and maintenance of tight junctions. Matrix metalloproteinase (MMP)-2 and -9 degrade type IV collagen of the extracellular matrix and basal membranes. Claudin-4 activates MMP-2, indicating that claudin-mediated increased cancer cell invasion may result from the activation of MMP proteins. In the present study, we used immunohistochemistry to examine the expression levels of claudin-4, MMP-2 and MMP-9 in 189 gastric cancer samples, and analyzed their correlation with tumor invasion, clinicopathologic parameters and clinical outcome. The relationship between claudin-4 expression and MMP-2 and -9 expression was also investigated. The expression of claudin-4 was found to be significantly higher in gastric cancer cases with advanced depth of wall invasion, lymph node metastasis, lymphatic invasion and higher TNM stage. Further analysis revealed claudin-4 expression to be significantly correlated with the expression of MMP-2 and -9. Kaplan-Meier survival analysis indicated that MMP-9 expression was correlated with poor prognosis. These results suggest that claudin-4 expression is associated with tumor invasion and with MMP-2 and -9 expression in gastric cancer. Additionally, MMP-9 expression was demonstrated to serve as a prognostic factor in patients with gastric cancer.

Introduction

Claudins are tight junctional proteins which are present at the epithelial and endothelial cell membranes (1,2), and are the major integral membrane proteins forming the backbone of tight junctions. Tight junctions form the primary barrier to the paracellular transport of solutes across cells, and also play a critical role in establishing and maintaining epithelial cell polarity (3,4). The claudin family consists of 23 transmembrane proteins exhibiting distinct tissue- and development-specific distribution patterns (5). The carboxylic terminal region of claudin proteins contains a PDZ domain-binding motif that potentially interacts with a number of PDZ domain-containing proteins, such as ZO proteins (6,7). These interactions also serve as adapters for other proteins involved in cell signaling. A number of other cytosolic and nuclear proteins, including regulatory proteins such as Rab3b, tumor suppressors such as PTEN and transcription factors such as ZONAB, also interact directly or indirectly with the tight junction complex (8-10). These interactions suggest that tight junctions, in addition to acting as barriers to the paracellular flow of solutes, may play an important role in regulating other cell functions, such as proliferation and tumor suppression.

Modulations in tight junction structure and function have been observed in epithelial tumorigenesis (11,12). A tissue microarray study showed that claudin-1, -3 and -4 are strongly expressed in most cases of intestinal-type gastric cancer, but are less frequently expressed in diffuse-type gastric cancer (13). Using cDNA microarray and immunohistochemical analysis, our group previously showed that the expression of claudin-4 was significantly higher in intestinal-type than in diffuse-type gastric cancer (14,15). Other studies have shown that claudin-2 expression gradually increases during the multistage process of gastric carcinogenesis (16,17). In addition, several studies have found aberrant claudin expression in various types of cancer, including increased expression of claudin-3 and -4 in prostate and uterine cancers (18,19), high claudin-4 expression in pancreatic cancer (20), down-regulation of claudin-7 in head and neck cancer (21) and metastatic breast cancer (22), and an increase in claudin-3 and -4 in breast cancer (23). However, the exact role of claudin overexpression and the functional importance of these proteins in the development of gastric cancer remain unclear.

Gastric cancer is one of the most common malignant tumors of the alimentary tract. At the time of diagnosis, it usually shows extensive local tumor invasion and frequent spread to metastatic sites, particularly the lymph nodes. It is thus characterized by late clinical presentation, rapid progression and a poor survival prognosis (24). The spread of malignant tumors is a multistep process, and many of the stages of tumor

Correspondence to: Dr Chi-Ming Wu, Department of Cosmetic Science, Vanung University, No. 1 Van-Nung Rd., Tao-Yuan 320, Taiwan, R.O.C.
E-mail: chimingwu@mail.vnu.edu.tw

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invasion require degradation or breakdown of the extracellular matrix and connective tissue surrounding tumor cells (25,26). The matrix metalloproteinases (MMPs) are a family of zinc-containing enzymes involved in the degradation of different components of the extracellular matrix. There is considerable evidence indicating that individual MMPs play crucial roles in tumor invasion and tumor spread (27-32). Some studies have suggested a major role for MMP-2 and -9 in the digestion of basement membrane type IV collagen as an important mechanism for vessal invasion and metastasis in gastric cancer (33,34).

Recent studies have indicated the modulatory effects of claudins on MMP activation. Agarwal *et al* showed that claudin-3 and -4 expression in ovarian epithelial cells enhanced invasion and was associated with increased MMP-2 activity (35). Oku *et al* showed that claudin-1 enhanced the invasive activity of oral squamous cell carcinoma cells by promoting the cleavage of the laminin-5 γ2 chain via MMP-2 and membrane-type MMP-1 (36). Takehara *et al* revealed that the overexpression of claudin-4 specifically stimulated the invasive activity of colonic cancer cells and increased MMP-2 and -9 activity (37). In the present study, we examined the expression levels of claudin-4, MMP-2 and -9 in gastric cancer in order to analyze their correlation with tumor invasion, clinicopathologic parameters and clinical outcome of gastric cancer patients. We also investigated the relationship between claudin-4 expression and MMP-2 and -9 expression in gastric cancer.

Materials and methods

Patients and specimens. A consecutive series of 189 tissue specimens was collected from patients with gastric cancer who underwent subtotal or total resection by gastrectomy at Chang Gung Memorial Hospital (CGMH), Taiwan between January 2001 and December 2002. Written informed consent was obtained before sample collection, and the study was approved by the Institutional Review Board of CGMH. The patients comprised 110 males and 79 females with a mean age of 62 years (range 24-90). The age and gender of the patients, tumor location, tumor size, cell differentiation, depth of wall invasion, status of lymph node metastasis, vascular invasion, lymphatic invasion and desmoplastic reaction were obtained from histopathology records. The stage of gastric cancer was described according to the 1997 tumor-node-metastasis (TNM) classification of malignant tumors of the American Joint Committee on Cancer. Follow-up was conducted until December 2007, for a minimum follow-up time of 5 years. The tissue specimens were formalin-fixed and paraffin-embedded, then stained with H&E and classified by a pathologist. The results were compared to histopathology records from CGMH. Final pathology was determined by consensus, with review if necessary.

Immunohistochemistry. Tissue blocks were constructed according to the method of Schraml *et al* (38), and the most representative morphological areas of the tumors were used in the study. The specimen sections were deparaffinized, treated with 3% hydrogen peroxide and microwaved after pre-treatment in 10 mM citric acid to retrieve antigenicity. The sections were incubated with blocking solution containing PBS and 1% bovine serum albumin for 20 min at room temperature, and then incubated overnight at 4˚C with an anti-claudin-4 antibody (1:100; Zymed, San Francisco, CA, USA), an anti-MMP-2 monoclonal antibody (1:50; Lab Vision Corporation, Fremont, CA, USA) and an anti-MMP-9 monoclonal antibody (1:50; Lab Vision Corporation), respectively. After washing 4 times with Tris-buffered saline, the sections were incubated with biotinylated secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The immunocomplex was visualized by the immonoglobulin enzyme bridge technique using the Dako LSAB 2 System, HRP kit (Dako Corp., Carpinteria, CA, USA) with 3,3'diaminobenzidine tetrachloride as a substrate. The sections were counterstained with hematoxylin, dehydrated with graded alcohol, cleared with xylene and mounted with coverslips.

Scoring of immunostaining. The results of immunostaining were scored according to a previous report (39) as follows: the immunostaining reaction was evaluated by subjective assessments of the median staining intensity (0, no stain; 1, weak; 2, moderate; 3, strong stain) and by the fraction of stained cells in percentage categories (0, 0-9%; 1, 10-49%; 2, 50-89%; and 3, ≥90%). This scoring system was previously shown to be reproducible (40). Scores of 0-3 were determined as follows: percentage categories and staining were each ranked as indicated above. The ranks for percentage and staining intensity were multiplied by each other, divided by 3 and rounded up to the nearest whole number (40). The results of immunostaining were classified as negative (whole number 0) or positive (whole number 1-3), respectively (Fig. 1).

Statistical analysis. The χ^2 test or Fisher's exact test were used to test for an association between claudin-4, MMP-2 and -9 expression and the clinicopathologic parameters of the patients. Disease-free survival was defined as the time from surgery to the first relapse of cancer, occurence of a second primary tumor or death of any cause. Univariate survival analysis was assessed by the Kaplan-Meier method, and the significance of differences between groups was analyzed using the log rank test or the log rank test for trend. Stepwise multivariate survival analysis was performed according to the Cox proportional hazards model. All reported P-values were two-sided, and P-values <0.05 were considered significant.

Results

Claudin-4, MMP-2 and MMP-9 expression in gastric cancer. Claudin-4 was expressed in the membrane of gastric adenocarcinoma cells in 84.7% (160/189) of cases. MMP-2 and -9 were expressed in the cytoplasm of gastric adenocarcinoma cells in 81% (153/189) and 89.4% (169/189) of the cases, respectively (Fig. 1).

Claudin-4, MMP-2 and MMP-9 expression in relation to clinicopathologic parameters. The expression of claudin-4 was significantly higher in males than in females (P=0.046), and was positively correlated with tumor size (P=0.008) and desmoplastic reaction (P=0.027). The expression of claudin-4 was significantly higher in gastric cancer with advanced depth of wall invasion (P=0.008), lymph node metastasis (P=0.005),

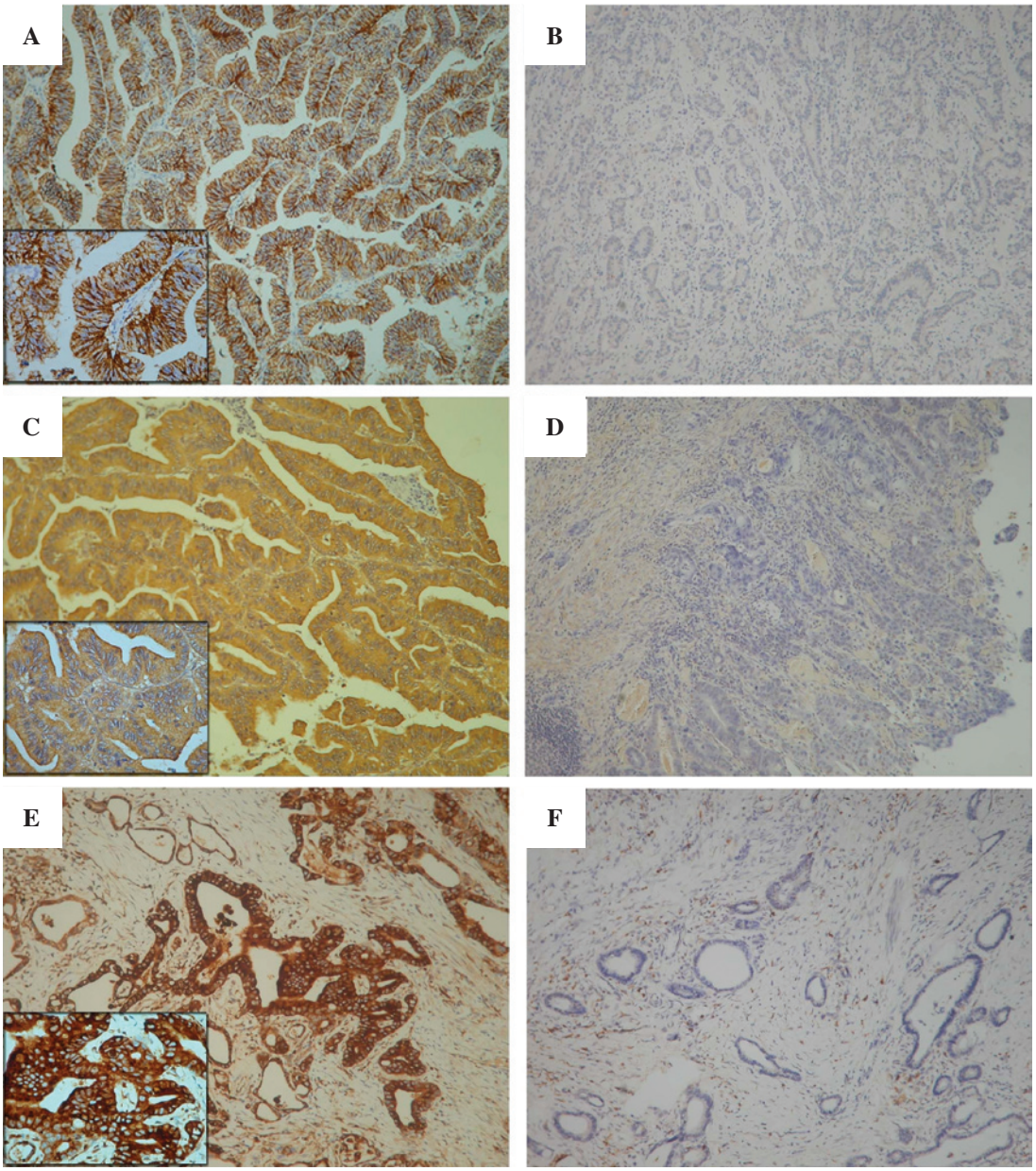

Figure 1. Immunohistochemistry of claudin-4, MMP-2 and MMP-9 in representative samples of gastric cancer. (A) Positive staining for claudin-4 in the cell membrane of gastric cancer cells. (B) Negative staining for claudin-4 in a gastric cancer specimen. (C) Positive staining for MMP-2 in the cytoplasm of gastric cancer cells. (D) Negative staining for MMP-2 in a gastric cancer specimen. (E) Positive staining for MMP-9 in the cytoplasm of gastric cancer cells. (F) Negative staining for MMP-9 in a gastric cancer specimen. Magnification x100. Inset shows immunohistochemistry at a high magnification (x400).

lymphatic invasion (P=0.001) and high TNM stage (P=0.004), but was not correlated with age, tumor location, cell differentiation or vascular invasion (Table I).

The expression of MMP-2 was significantly higher in males than in females (P=0.036), and was significantly correlated with tumor location (P=0.01), tumor size (P=0.024), cell differentiation (P=0.04) and desmoplastic reaction (P=0.021), but not with age, depth of wall invasion, lymph node metastasis, vascular invasion, lymphatic invasion or TNM stage (Table I).

MMP-9 expression was positively correlated with tumor size (P=0.004) and desmoplastic reaction (P=0.02). As with claudin-4, the expression of MMP-9 was significantly higher in gastric cancer with advanced depth of wall invasion (P=0.002), lymph node metastasis (P=0.038), lymphatic invasion (P=0.046) and higher TNM stage (P=0.008), but was not correlated with age, gender, tumor location, cell differentiation or vascular invasion (Table I).

Correlation of claudin-4 expression with MMP-2 and MMP-9 expression. Further analysis of the relationship between claudin-4 expression and MMP-2 and -9 expression revealed claudin-4 expression to be significantly correlated with the expression of these two proteins (P=0.005 and 0.018, respectively; Table II). To better define the pattern of co-expression between claudin-4 and these two proteins, immunostaining was conducted in serial sections of gastric cancer. Of 189 specimens, 135 (71.4%) were positive for claudin-4- and MMP-2, and 147 (77.8%) were positive for claudin-4- and MMP-9 (Table II and Fig. 2).

Table I. Association of claudin-4, MMP-2 and MMP-9 expression with clinicopathologic parameters.

Factors	Cases	Claudin-4 expression			MMP-2 expression			MMP-9 expression		
		Negative n (%)	Positive n (%)	P-value	Negative n (%)	Positive n (%)	P-value	Negative n (%)	Positive n (%)	P-value
		n=29	n=160		n=36	n=153		n=20	n=169	
Age (years)										
≤60	81	15 (51.7)	66 (41.3)	0.294	18 (50.0)	63 (41.2)	0.336	9 (45.0)	72 (42.6)	0.838
>60	108	14 (48.3)	94 (58.8)		18 (50.0)	90 (58.8)		11 (55.0)	97 (57.4)	
Gender										
Male	110	12 (41.4)	98 (61.2)	0.046	16 (44.4)	94 (61.4)	0.036	8 (40.0)	102 (60.4)	0.081
Female	79	17 (58.6)	62 (38.8)		20 (55.6)	59 (38.6)		12 (60.0)	67 (39.6)	
Tumor location										
Lesser curvature lesion	40	7 (24.1)	33 (20.6)	0.554	14 (38.9)	26 (17.0)	0.01	4 (20.0)	36 (21.3)	0.569
Prepyloric	121	20 (69.0)	101 (63.1)		21 (58.3)	100 (65.4)		15 (75.0)	106 (62.7)	
Proximal cardioesophageal	21	2 (6.9)	19 (11.9)		1 (2.8)	20 (13.1)		1 (5.0)	20 (11.8)	
Diffuse	7	0 (0.0)	7 (4.4)		0 (0.0)	7 (4.6)		0 (0.0)	7 (4.1)	
Tumor size (cm)										
≤3	94	21 (72.4)	73 (45.6)	0.008	24 (66.7)	70 (45.8)	0.024	16 (80.0)	78 (46.2)	0.004
>3	95	8 (27.6)	87 (54.4)		12 (33.3)	83 (54.2)		4 (20.0)	91 (53.8)	
Differentiation										
Well	18	1 (3.4)	17 (10.6)	0.226	0 (0.0)	18 (11.8)	0.04	0 (0.0)	18 (10.7)	0.153
Moderate	53	6 (20.7)	47 (29.4)		8 (22.2)	45 (29.4)		4 (20.0)	49 (29.0)	
Poor	118	22 (75.9)	96 (60.0)		28 (77.8)	90 (58.8)		16 (80.0)	102 (60.3)	
Depth of wall invasion										
T1	47	14 (48.3)	33 (20.6)	0.008	14 (38.9)	33 (21.6)	0.151	12 (60.0)	35 (20.7)	0.002
T2	35	5 (17.2)	30 (18.8)		7 (9.4)	28 (18.3)		3 (15.0)	32 (18.9)	
T3	92	10 (34.5)	82 (51.3)		13 (6.1)	79 (51.6)		4 (20.0)	88 (52.1)	
T4	15	0 (0.0)	15 (9.4)		2 (5.6)	13 (8.5)		1 (5.0)	14 (8.3)	
Lymph node metastasis										
N0	86	22 (75.9)	64 (40.0)	0.005	22 (61.1)	64 (41.8)	0.186	15 (75.0)	71 (42.0)	0.038
N1	44	3 (10.3)	41 (25.6)		5 (13.9)	39 (25.5)		2 (10.0)	42 (24.9)	
N2	22	1 (3.4)	21 (13.1)		4 (11.1)	18 (11.8)		2 (10.0)	20 (11.8)	
N3	37	3 (10.3)	34 (21.3)		5 (13.9)	32 (20.9)		1 (5.0)	36 (21.3)	
Vascular invasion										
No	165	28 (96.6)	137 (85.6)	0.134	34 (94.4)	131 (85.6)	0.263	0 (0.0)	24 (14.2)	0.082
Yes	24	1 (3.4)	23 (14.4)		2 (5.6)	22 (14.4)		20 (100.0)	145 (85.8)	

Table I. Continued.

Factors	Cases	Claudin-4 expression			MMP-2 expression			MMP-9 expression		
		Negative	Positive	P-value	Negative	Positive	P-value	Negative	Positive	P-value
Lymphatic invasion										
No	102	24 (82.8)	78 (48.8)	0.001	22 (61.1)	80 (52.3)	0.339	5 (25.0)	82 (48.5)	0.046
Yes	87	5 (17.2)	82 (51.2)		14 (38.9)	73 (47.7)		15 (75.0)	87 (51.5)	
Desmoplastic reaction										
None	29	9 (31.0)	20 (12.5)	0.034	11 (30.6)	18 (11.8)	0.028	5 (25.0)	24 (14.2)	0.021
Mild	62	11 (37.9)	51 (31.9)		11 (30.6)	51 (33.3)		11 (55.0)	51 (30.2)	
Moderate	73	7 (24.1)	66 (41.3)		12 (33.3)	61 (39.7)		4 (20.0)	69 (40.8)	
Marked	25	2 (6.9)	23 (14.4)		2 (5.6)	23 (15.0)		0 (0.0)	25 (14.8)	
TNM stage										
I	67	18 (62.1)	49 (30.6)	0.004	22 (61.1)	64 (41.8)	0.186	14 (70.0)	53 (31.4)	0.008
II	37	6 (20.7)	31 (19.4)		5 (13.9)	39 (25.5)		1 (5.0)	36 (21.3)	
III	37	1 (3.4)	36 (22.5)		4 (11.1)	18 (11.8)		2 (10.0)	35 (20.7)	
IV	48	4 (13.8)	44 (27.5)		5 (13.9)	32 (20.9)		3 (15.0)	45 (26.6)	

Table II. Association of claudin-4 expression with MMP-2 and MMP-9 expression.

Factors	Claudin-4 expression		
	Negative n (%)	Positive n (%)	P-value
	n=29	n=160	
MMP-2 expression			
Negative (-)	11 (37.9)	25 (15.6)	0.005
Positive (+)	18 (62.1)	135 (84.4)	
MMP-9 expression			
Negative (-)	7 (24.1)	13 (8.1)	0.018
Positive (+)	22 (75.9)	147 (91.9)	

Prognostic implications of claudin-4, MMP-2 and MMP-9 expression in gastric cancer. MMP-9 expression was correlated with a poor prognosis (P=0.041; Table III and Fig. 3C). Neither claudin-4 nor MMP-2 expression was correlated with survival (Table III, Fig. 3A and B). Other prognostic factors were type of gastrectomy, tumor location, large tumor size, poor cell differentiation, advanced penetration depth, presence of nodal metastases, presence of vascular or lymphatic invasion, marked desmoplastic reaction and higher stage. In multivariate analysis, depth of invasion, lymph node metastasis and lymphatic invasion were independent prognostic factors (Table IV).

Discussion

In this study, claudin-4, MMP-2 and MMP-9 expression was examined in 189 cases of gastric cancer, and was associated with patient clinicopathologic factors. Claudin-4 expression was correlated with depth of wall invasion, lymph node metastasis and lymphatic invasion, and was significantly correlated with MMP-2 and -9 expression. These results are consistent with those obtained in a cancer cell model (35,37). Agarwal *et al* showed that claudin-4 expression in ovarian epithelial cells enhanced cell invasion and was associated with increased MMP-2 activity (35). Takehara *et al* also showed that the overexpression of claudin-4 in colonic cancer cells stimulated invasive activity and MMP-2 and -9 activity (37). Although it is generally believed that an alteration in claudin expression is involved in tumorigenesis, the role of claudin-4 in the regulation of cancer-related cell functions, such as invasion, remains unclear. It is known that claudins affect cell physiology by recruiting signal transduction-related molecules at tight junctions. Claudin-4 affects the expression and activity of MMP-2 and -9 either directly or by modulating signal transduction; consequently, these two proteins stimulate cell invasion.

Recent studies also indicate that the overexpression of claudins is correlated with tumor invasion. Wu *et al* demonstrated that the overexpression of claudin-1 was correlated with the invasiveness and metastasis of gastric cancer (41).

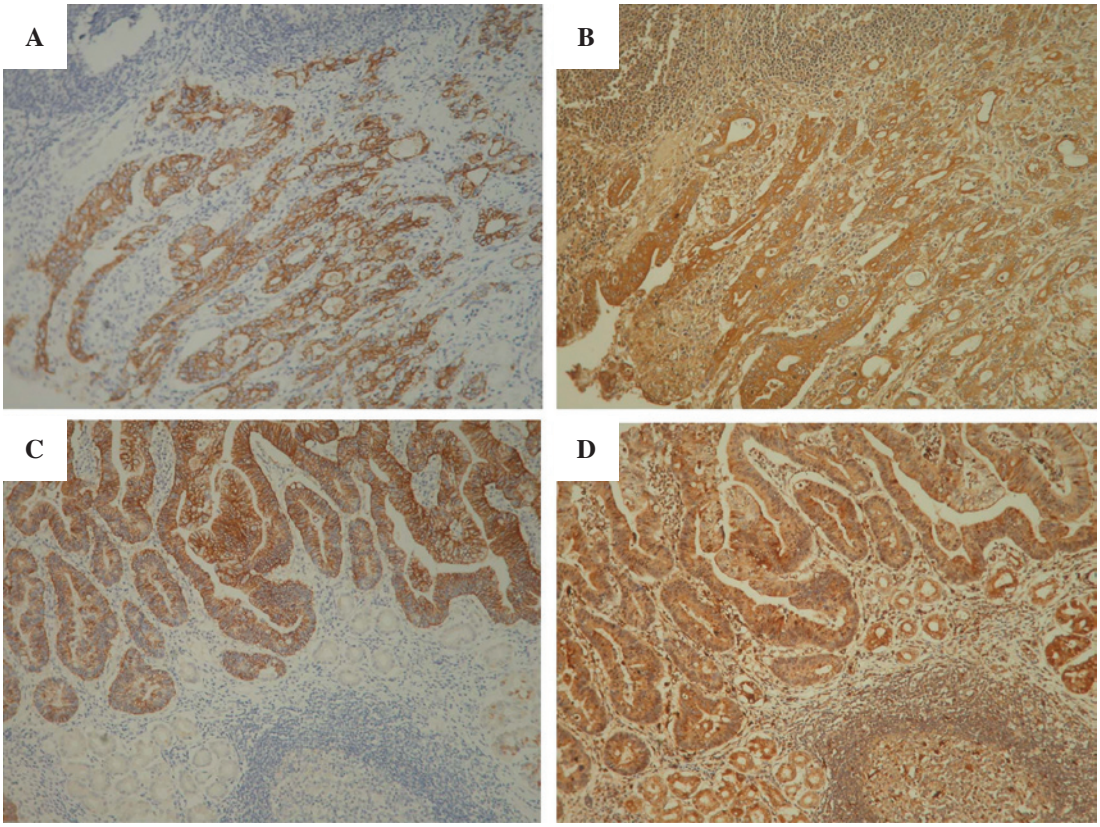

Figure 2. Immunohistochemical analysis using serial sections of gastric cancer showing co-expression of claudin-4 (A), MMP-2 (B), claudin-4 (C) and MMP-9 (D) in gastric cancer. Magnification x100.

Kinugasa *et al* revealed that the expression of claudin-1 and -2 was up-regulated in colorectal cancer, and that this up-regulation was correlated with the depth of tumor invasion (42). Dhawan *et al* showed that claudin-1 expression increased with the progression of colon carcinoma and metastasis (43). Nevertheless, some studies have shown that the down-regulation of claudins is correlated with tumor invasion. Ueda *et al* showed that decreased claudin-4 expression at the invasive front is correlated with cancer invasion and metastasis in colorectal cancer (44). Oshima *et al* showed that reduced expression of claudin-7 correlated with venous invasion and liver metastasis in colorectal cancer (45). Usami *et al* reported that reduced expression of claudin-7 at the invasive front of esophageal squamous cell carcinoma may lead to tumor progression and subsequent metastasis (46). Morohashi *et al* found that decreased expression of claudin-1 correlated with lymphatic node metastasis in breast cancer (47). These reports of decreased claudin protein expression in cancer are consistent with the generally accepted notion that tumorigenesis is accompanied by a disruption of the tight junctions, a process that may play a key role in the loss of cohesion and invasiveness observed in cancer cells.

In gastric cancer, MMP-2 and -9 are linked to tumor invasion and metastasis as well as to a poor prognosis (48-50). Kabashima *et al* demonstrated a correlation between MMP-9 expression and lymphatic invasion and lymph node positivity in gastric carcinoma (51). The results of Kabashima *et al* are consistent with the results of the present study, which indicate that MMP-9, but not MMP-2 expression, is positively correlated with lymph node metastasis and lymphatic invasion as well as with a poor prognosis. By contrast, certain reports have shown MMP-2 expression to be associated with tumor invasion, lymph node metastasis and survival in gastric cancer (52-54). Allgyer *et al* demonstrated an association between the immunohistochemical detection of MMP-2 and the prognosis of gastric cancer patients (52). Ji *et al* showed that the expression of MMP-2 mRNA was significantly correlated with lymph node metastasis and a poor prognosis in gastric cancer patients (53). Monig *et al* also found that the intensity of MMP-2 staining in tumor cells was significantly correlated with the depth of tumor infiltration, lymph node metastasis and distal metastasis in gastric cancer patients (54). Further studies are required to clearly distinguish the roles and involvement of MMP-2 and -9 in the metastasis of gastric cancer.

In conclusion, claudin-4 expression was correlated with the depth of wall invasion, lymph node metastasis and lymphatic invasion in gastric cancer. Further analysis showed that claudin-4 expression was significantly correlated with MMP-2 and -9 expression. We suggest that claudin-4 affects the expression and activity of MMP-2 and -9 either directly or by modulating signal transduction, and that these two proteins stimulate tumor cell invasion.

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Table III. Univariate analysis of the clinicopathologic parameters influencing disease-free survival in 189 gastric cancer patients undergoing gastrectomy.

Factors	No. cases	Mean survival (months)	95% CI of mean	5-year survival (%)	P-value
Age (years)					
≤60	81	54.6	46.9-62.3	58.8	0.7750
>60	108	54.9	48.1-61.7	61.2	
Gender					
Male	110	58.5	52.0-65.0	65.1	0.0820
Female	79	49.6	41.6-57.7	53.3	
Type of gastrectomy					
Total	42	29.3	20.2-38.4	33.8	<0.0001
Subtotal	147	60.8	55.4-66.1	67.7	
Tumor location					
Lesser curvature lesion	40	65.9	56.7-75.1	74.5	<0.0001
Prepyloric	121	56.7	50.4-62.9	62.3	
Proximal cardioesophageal	21	24.8	13.2-36.4	30.2	
Diffuse	7	22.9	4.5-41.3	28.6	
Tumor size (cm)					
≤3	94	72.2	67.2-77.1	83.4	<0.0001
>3	95	36.9	29.5-44.2	36.0	
Differentiation					
Well	18	65.4	59.0-71.9	94.4	0.0190
Moderate	53	43.7	35.2-52.2	58.1	
Poor	118	52.6	46.1-59.0	51.5	
Depth of invasion					
T1	47	79.4	75.0-83.7	95.7	<0.0001
T2	35	74.2	67.2-81.3	85.4	
T3	92	39.3	32.1-48.5	38.9	
T4	15	8.2	4.6-11.7	0.0	
Lymph node metastasis					
N0	86	74.9	70.6-79.3	86.6	<0.0001
N1	44	60.2	50.0-70.4	69.6	
N2	22	27.2	16.1-38.3	26.0	
N3	37	11.2	7.0-15.4	0.0	
Vascular invasion					
No	165	60.9	55.9-66.0	68.1	<0.0001
Yes	24	11.1	5.4-16.9	4.5	
Lymphatic invasion					
No	102	71.3	66.5-76.2	81.5	<0.0001
Yes	87	34.7	27.1-42.4	34.3	
Desmoplastic reaction					
None	29	67.4	56.5-78.2	79.3	<0.0001
Mild	62	73.3	67.7-79.0	84.4	
Moderate	73	38.1	30.0-46.2	37.9	
Marked	25	34.5	21.3-47.8	39.5	
TNM stage					
I	67	79.4	76.3-82.6	93.9	<0.0001
II	37	70.1	61.8-78.4	78.9	
III	37	44.8	33.5-56.0	47.6	
IV	48	10.4	6.9-13.9	0.0	
Claudin-4					
Negative	29	64.2	53.0-75.3	71.5	0.1690
Positive	160	53.0	47.3-58.6	58.0	
MMP-2					
Negative	36	63.0	53.5-72.6	71.5	0.1120
Positive	153	52.4	46.6-58.2	57.4	

Table III. Continued

Factors	Cases	Mean survival (months)	95% CI of mean	5-year survival (%)	P-value
MMP-9					
Negative	20	68.3	56.0-80.7	84.2	0.0410
Positive	169	52.9	47.5-58.3	57.2	

CI, confidence interval.

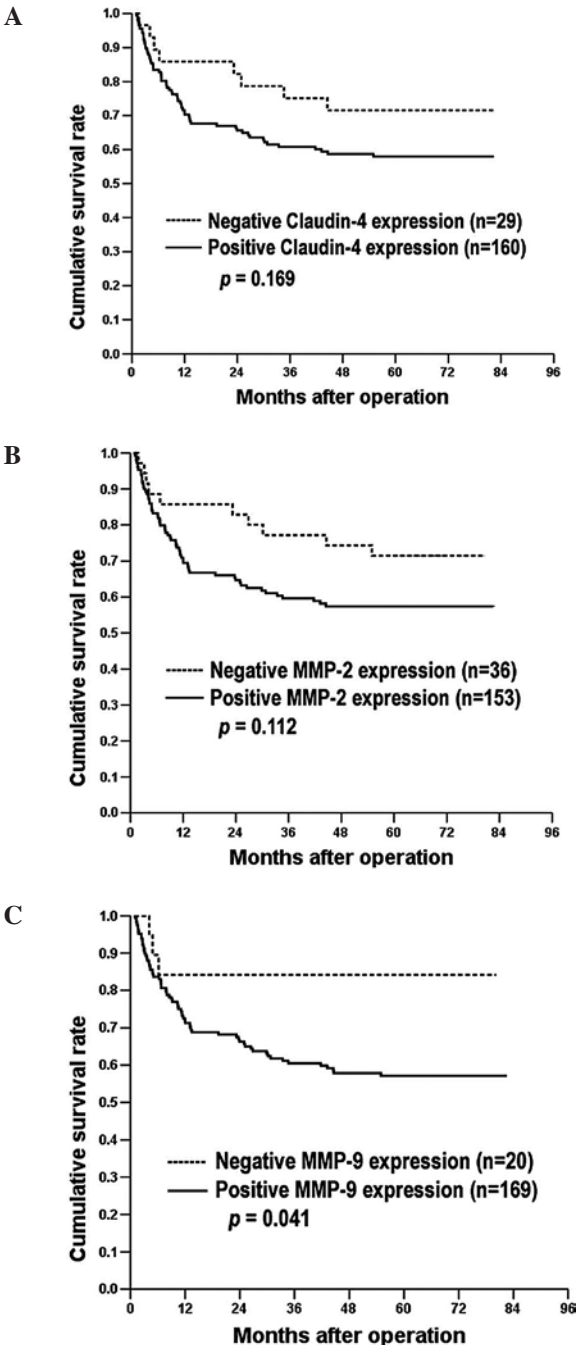

Figure 3. Kaplan-Meier survival curves for disease-free survival of 189 patients with gastric cancer. (A) Categorized by claudin-4 expression, no significant difference was observed between patients with positive claudin-4 expression and those with negative (P=0.169). (B) Categorized by MMP-2 expression, no significant difference was observed between the two groups (P=0.112). (C) Categorized by MMP-9 expression, survival was significantly better for patients with negative MMP-9 expression than for those with positive (P=0.041).

Table IV. Multivariate Cox's proportional hazards analysis for disease-free survival of 189 gastric cancer patients undergoing gastrectomy.

Factors	Relative risk (95% CI)		P-value
Depth of invasion			<0.0001
T2 vs. T1	5.543	(0.962-31.950)	0.0550
T3 vs.T1	20.420	(4.023-103.659)	0.0003
T4 vs. T1	35.392	(6.037-207.466)	<0.0001
Lymph node metastasis			0.0005
N1 vs. N0	1.926	(0.831-4.465)	0.1270
N2 vs. N0	3.643	(1.516-8.756)	0.0040
N3 vs. N0	5.779	(2.387-13.990)	0.0001
Lymphatic invasion			
Yes vs. no	2.115	(1.188-3.766)	0.0110

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