Histol Histopathol (2008) 23: 515-521

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Expression of matrix metalloproteinases MMP-2 and MMP-9 in gastric cancer and their relation to claudin-4 expression

Li-Yu Lee[1,2], Chi-Ming Wu[3], Chee-Chan Wang[3], Jau-Song Yu[4], Ying Liang[4], Kuo-Hao Huang[4], Chih-Hong Lo[5] and Tsann-Long Hwang[5]

[1]Department of Pathology, Chang Gung Memorial Hospital, Tao-Yuan, Taiwan, [2]Graduate Institute of Clinical Medical Sciences, Chang Gung University, Tao-Yuan, Taiwan, [3]Department of Cosmetic Science, Vanung University, Tao-Yuan, Taiwan, [4]Graduate Institute of Basic Medical Science, Chang Gung University, Tao-Yuan, Taiwan and [5]Department of Surgery, Chang Gung Memorial Hospital, Tao-Yuan, Taiwan

* Li-Yu Lee and Chi-Ming Wu contributed equally to this work.

Summary. Matrix metalloproteinases (MMPs) MMP-2 and MMP-9 can degrade type IV collagen of extracellular matrix and basal membranes. Claudin-4 is a member of a large family of transmembrane proteins, claudins, essential in the formation and maintenance of tight junctions. Claudin-4 has been shown to activate MMP-2, indicating that claudin-mediated increased cancer cell invasion might be mediated through the activation of MMP proteins. To explore the roles of MMP-2, MMP-9 and claudin-4 in gastric cancer, we selected 88 cases and then analyzed the expression of these proteins using immunohistochemistry. We found that all of MMP-2, MMP-9 and claudin-4 expressions were significantly higher in intestinal-type than in diffuse-type gastric cancer. On further analysis, testing the relationship between MMP-2 and MMP-9 expression with claudin-4 expression, claudin-4 expression was significantly associated with MMP-9 expression, but not with MMP-2 expression. The results showed that MMP-2, MMP-9 and claudin-4 expression may be phenotypic features, distinguishing intestinal-type and diffuse-type gastric cancer. Possibly, claudin-4 played a role in determining MMP-9 activity which favored intestinal-type gastric cancer to distal metastasis.

Offprint requests to: Tsann-Long Hwang, Department of Surgery, Chang Gung Memorial Hospital, No.5 Fu-Hsing Street, 333 Tao-Yuan, Taiwan. e-mail: hwangtl@adm.cgmh.org.tw

Key words: Claudin-4, Matrix metalloproteinases, MMP-2, MMP-9, Gastric cancer

Introduction

Gastric cancer is one of the commonest malignant tumors of the alimentary tract and is characterized by late clinical presentation, rapid progression, and poor survival (Morson et al., 1990). The reason for this poor prognosis is that, at the time of diagnosis, gastric cancer usually shows extensive local tumor invasion and frequent spread to metastatic sites, particularly lymph nodes. Spread of malignant tumors is a multistep process and many of the stages of tumor invasion require degradation or breakdown of the extracellular matrix and connective tissue surrounding tumor cells (Hart and Saini, 1992; Kohn and Liotta, 1995). The matrix metalloproteinases (MMPs) are a family of zinc containing enzymes which are involved in the degradation of different components of the extracellular matrix, and there is considerable evidence to indicate that individual MMPs have important roles in tumor invasion and tumor spread (Murphy and Docherty, 1992; Boag and Young, 1994; Urbanski et al., 1993). Recent studies have suggested a major role for MMP-2 and MMP-9 in the digestion of basement membrane type IV collagen, as an important mechanism for vessal invasion and metastasis in gastric cancer (Sakurai et al., 1997; Torii et al., 1997).

Claudins are a large family of transmembrane

proteins essential in the formation and maintenance of tight junctions (Gonzalez-Mariscal et al., 2003). Tight junctions in epithelial cells provide a selective barrier and establish cellular polarity (Mitic et al., 2000; Tsukita et al., 2001; Matter and Balda, 2003). These structures are typically lost in cancer and this loss may contribute to the invasive and metastatic phenotype of tumor cells (Martin and Jiang, 2001; Itoh and Bissell, 2003; Langbein et al., 2003; Mullin, 2004). Using cDNA microarray and immunohistochemistry analysis, we have previously shown that *claudin-4* was more overexpressed in intestinal-type than in diffuse-type gastric cancer (Kuo et al., 2006; Wu et al., 2006). We found overexpression of claudin-4 in intestinal-type, and, in general, liver metastasis were frequently seen in intestinal-type gastric cancer (Umehara et al., 1992). Overexpression of claudin-4 may be associated with the distal metastasis of gastric cancer. A recent report showed that claudin-4 expression was associated with increased MMP-2 activity in ovarian epithelial cells and enhanced cell invasion (Agarwal et al., 2005).

The carboxylic terminal region of claudin proteins contains a PDZ domain-binding motif that can potentially interact with a number of PDZ domain-containing proteins, such as ZO proteins (Itoh et al., 1999; Morita et al., 1999). These interactions can also serve as adapters for other proteins involved in cell signaling. A number of other cytosolic and nuclear proteins, which include regulatory proteins Rab3b, tumor suppressors like PTEN and transcription factors like ZONAB, have also been shown to interact directly or indirectly with tight junction complex (Wu et al., 2000; Balda et al., 2003; Yamamoto et al., 2003). These interactions suggest that tight junctions, in addition to acting as barriers to paracellular flow of solutes, may play an important role in regulating other cell functions, such as proliferation and cell invasion.

In the present study, we used 88 gastric cancer specimens to examine expression of MMP-2, MMP-9 and claudin-4, and correlated these two MMPs expression with claudin-4 expression.

Materials and methods

Patients and specimens

A consecutive series of 88 tissue specimens were collected from patients with gastric cancer requiring subtotal or total gastroectomy resection in Chang Gung Memorial Hospital (CGMH) in Taiwan. All operations were performed between January 2000 and December 2001. Written informed consent was obtained before collection and this study was approved by the Institutional Review Board. There were 52 males and 36 females with a mean age of 60 years (range, 26-82 years). The age and gender of patients, depth of wall invasion, status of lymph node metastasis, cell differentiation and histological type were obtained from histopathology records. All tissue specimens were formalin-fixed and paraffin-embedded. Formalin fixed tissue sections were stained with H&E and classified by a pathologist. These results were compared with the histopathology records from CGMH. Final pathology was determined by consensus and review if necessary.

Immunohistochemistry

The tissue blocks were constructed according to the method of Schraml et al. (2003) and the best representative morphological areas of tumors were used in this study. The specimen sections were deparaffinized, treated with 3% hydrogen peroxide and microwaved after pretreatment 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-MMP-2 monoclonal antibody (1:50, Lab Vision Corporation, Fremont, CA), an anti-MMP-9 monoclonal antibody (1:50, Lab Vision Corporation) and an anti-claudin-4 antibody (1:100, Zymed, San Francisco, CA), respectively. After washing 4 times with TBS, the sections were incubated with biotinylated secondary antibody (Santa Cruz Biotechnology). The immuno-complex was visualized by the immonoglobulin enzyme bridge technique using the DAKO LSAB 2 System, HRP kit (DAKO corp. Carpinteria, CA) with 3,3' diaminobenzidine tetrachloride as a substrate. The sections were lightly counterstained with hematoxylin, dehydrated with graded alcohols, cleared with xylene and mounted with a coverslip.

Scoring of the immunohistochemical staining

These three proteins' immunostaining results were scored as follows, according to a previous report (Ravn et al., 1998). The immunostaining reaction was evaluated by subjective assessments of the median staining intensity (0, no stain; 1, weak; 2, moderate; and 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 (Ravn et al., 1993). The scores of 0 to 3 were obtained 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 (Ravn et al., 1993). The results of immunostaining were classified as negative (whole number 0) or positive (whole number 1-3), respectively (Fig. 1).

Statistical analysis

Chi-squared or Fisher's exact test was used to test for an association between MMP-2, MMP-9, claudin-4 expression and patients' clinicopathological parameters. The level of significance was set at 0.05. All reported P

values were two-sided. All data analyses were carried out using the SAS statistics package (version 8.1 for windows; SAS Institute, Inc., Cary, NC).

Results

The tumor specimens of 88 cases were assayed for MMP-2, MMP-9 and claudin-4 expression by performing immunohistochemical staining. For MMP-2 expression, 53 (60.2%) cases had a positive expression and 35 (39.8%) cases had a negative expression (Fig. 1A,B and Table 1). For MMP-9, 60 (68.2%) cases had a positive expression and 28 (31.8%) cases had a negative expression (Fig. 1C,D and Table 1). For claudin-4, 62 (70.5%) cases had a positive expression and 26 (29.5%) cases had a negative expression (Fig. 1E,F and Table 1).

We tested the correlation between MMP-2, MMP-9 and claudin-4 expression and such clinicopathological parameters as age, gender, histological type, depth of wall invasion, lymph node metastasis and cell differentiation (Table 1). For MMP-2, expression of MMP-2 was associated with histological type (positive expression, intestinal-type versus diffuse-type: 62.3% versus 37.7%, P=0.001). Except for histological type, MMP-2 expression was not associated the other parameters. For MMP-9, expression of MMP-9 was also associated with histological type (positive expression, intestinal-type versus diffuse-type: 63.3% versus 36.7%, P≤0.001). Except for histological type, MMP-9 expression was not associated with the other parameters, but a trend was observed between MMP-9 expression and cell differentiation (P=0.093). For claudin-4, expression of claudin-4 was also associated with histological type (positive expression, intestinal-type versus diffuse-type: 62.9% versus 37.1%, P≤0.001). Except for histological type, claudin-4 expression was not associated with the other parameters, but a trend was observed between claudin-4 expression and lymph node metastasis (P=0.085).

On further analysis for testing the relationship between MMP-2 and MMP-9 expression with claudin-4 expression, claudin-4 expression was significantly associated with MMP-9 expression, but not with MMP-2 expression (P<0.001 and P=0.204, respectively, Table 2).

Table 1. Association of MMP-2, MMP-9 and claudin-4 expression with the clinicopathological parameters.

Factor	Cases	MMP-2 expression			MMP-9 expression			claudin-4 expression		
		Negative n=35 (%)	Positive n=53 (%)	P-value	Negative n=28 (%)	Positive n=60 (%)	P-value	Negative n=26 (%)	Positive n=62 (%)	P-value
Age										
≤ 60	44	19 (54.3%)	25 (47.2%)	0.513	19 (67.9%)	25 (41.7%)	0.221	16 (61.5%)	28 (45.2%)	0.161
>60	44	16 (45.7%)	28 (52.8%)		9 (32.1%)	35 (58.3%)		10 (38.5%)	34 (54.8%)	
Gender										
Male	52	18 (51.4%)	34 (64.2%)	0.235	17 (60.7%)	35 (58.3%)	0.832	16 (61.5%)	36 (58.1%)	0.762
Female	36	17 (48.6%)	19 (35.8%)		11 (39.3%)	25 (41.7%)		10 (38.5%)	26 (41.9%)	
Histological type										
Intestinal	42	9 (25.7%)	33 (62.3%)	0.001	4 (14.3%)	38 (63.3%)	<0.001	3 (11.5%)	39 (62.9%)	<0.001
Diffuse	46	26 (74.3%)	20 (37.7%)		24 (85.7%)	22 (36.7%)		23 (88.5%)	23 (37.1%)	
Depth of wall invasion										
T1	23	13 (37.1%)	10 (18.9%)	0.144	5 (17.9%)	18 (30.0%)	0.385	9 (34.6%)	14 (22.6%)	0.692
T2	10	2 (5.7%)	8 (15.1%)		5 (17.9%)	5 (8.3%)		3 (11.5%)	7 (11.3%)	
T3	43	17 (48.6%)	26 (49.1%)		15 (53.6%)	28 (46.7%)		11 (42.3%)	32 (51.6%)	
T4	12	3 (8.6%)	9 (16.9%)		3 (10.6%)	9 (15.0%)		3 (11.6%)	9 (14.5%)	
Lymph node metastasis										
-	32	16 (45.7%)	16 (30.2%)	0.138	12 (42.9%)	20 (33.3%)	0.387	13 (50.0%)	19 (30.6%)	0.085
+	56	19 (54.3%)	37 (69.8%)		16 (57.1%)	40 (66.7%)		13 (50.0%)	43 (69.4%)	
Differentiation										
Well	9	5 (14.3%)	4 (7.5%)	0.419	2 (7.1%)	7 (11.7%)	0.093	1 (3.8%)	8 (12.9%)	0.406
Moderate	31	10 (28.6%)	21 (39.6%)		6 (21.4%)	25 (41.7%)		9 (34.6%)	22 (35.5%)	
Poor	48	20 (57.1%)	28 (52.9%)		20 (71.4%)	28 (46.6%)		16 (61.5%)	32 (51.6%)	

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

Factor	MMP-2 expression			MMP-9 expression		
	Negative n=35 (%)	Positive n=53 (%)	P-value	Negative n=28 (%)	Positive n=60 (%)	P-value
Claudin-4 expression						
Negative (–)	13 (37.1%)	13 (24.5%)	0.204	16 (57.1%)	10 (16.7%)	<0.001
Positive (+)	22 (62.9%)	40 (75.5%)		12 (42.9%)	50 (83.3%)	

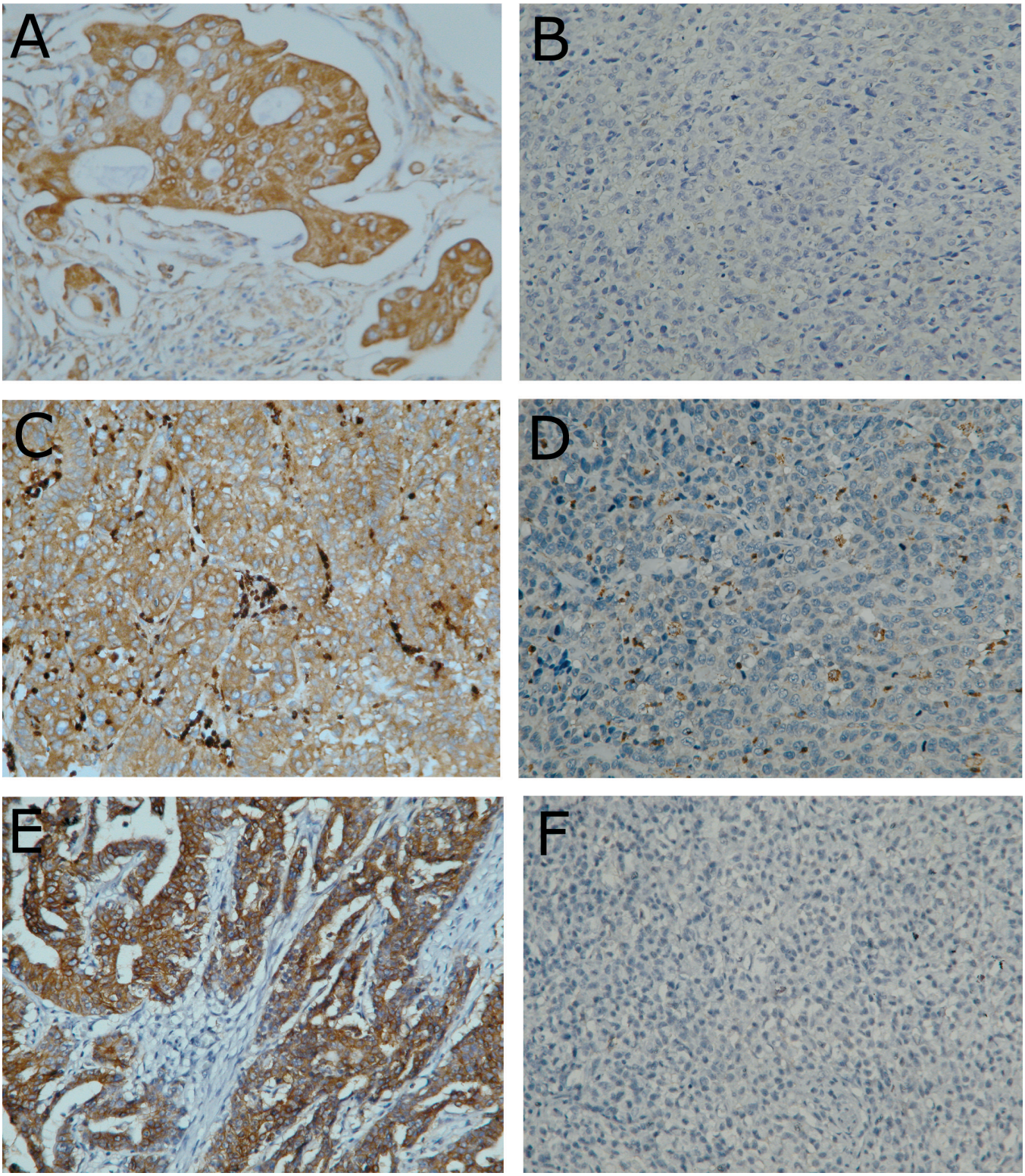

Fig. 1. Immunohistochemistry of MMP-2, MMP-9 and claudin-4 in gastric cancer. **A.** MMP-2 staining is positive in the cytoplasm of intestinal-type gastric cancer cells. **B.** MMP-2 staining is negative in diffuse-type gastric cancer. **C.** MMP-9 staining is positive in the cytoplasm of intestinal-type gastric cancer cells. **D.** MMP-9 staining is negative in diffuse-type gastric cancer. **E.** claudin-4 staining is positive in the cell membrane of intestinal-type gastric cancer cells. **F.** claudin-4 staining is negative in diffuse-type gastric cancer. x 200

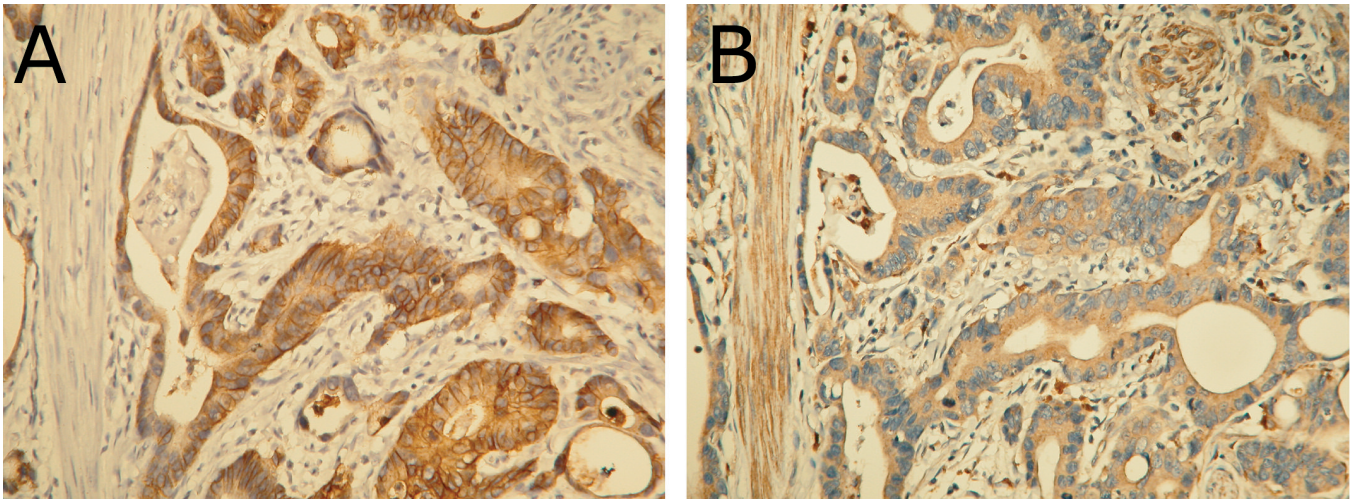

Fig. 2. Claudin-4 and MMP-9 co-expression in serial sections of gastric cancer. **A.** Claudin-4 staining is positive in the cell membrane of gastric cancer cells. **B.** MMP-9 staining is positive in the cytoplasm of gastric cancer cells. x 200

To better define the pattern of co-expression between claudin-4 and MMP-9, serial sections of gastric cancer were performed in 50 cases (Table 2) previously shown to be both claudin-4 and MMP-9 positive (Fig. 2).

Discussion

In this study, MMP-2, MMP-9 and claudin-4 expression were studied in 88 cases of gastric cancer and associated with the patients' clinicopathological factors. MMP-2 and MMP-9 expression were correlated with intestinal-type of tumor. Gastric cancer has been classified into two major types: intestinal- and diffuse-type (Lauren, 1965). Intestinal-type has a distinct glandular formation, while diffuse-type is characterized by isolation of cancer cells, infiltrating growth pattern and deposition of a large amount of collagen fibers. It is generally accepted that each histological type exhibits a different biological behavior. Intestinal-type tends to metastasize via both hematogenous and lymphatic routes, while diffuse-type tends to cause peritoneal dissemination or lymph node metastasis with rare hematogenous metastasis (Sugano et al., 1982). The mechanisms of tumor progression are different between the two types. In concordance with our results, Murray et al. reported that MMP-9 is correlated with intestinal-type gastric cancer (Murray et al., 1998) and Mrena et al. also showed that epithelial MMP-9 immunoreactivity correlated with intestinal-type gastric cancer (Mrena et al., 2006).

The results of claudin-4 expression in gastric cancer depicted that diffuse-type had lower expression compared with intestinal-type. Thus, loss of claudin-4 expression may be one phenotypic feature distinguishing these two tumor types, in analogy with E-cadherin, the expression of which was also lost in diffuse-type gastric cancer. We previously showed that overexpression of claudin-4 in intestinal-type gastric cancer (Kuo et al., 2006), and, in general , liver metastasis were frequently seen in this type of tumor (Umehara et al., 1992). The overexpression of claudin-4 may be associated with the distal metastasis of gastric cancer. Otherwise, the lower expression of claudin-4 in diffuse-type correlated well with the general observation that peritoneal metastasis were frequently seen in this type of tumor (Umehara et al., 1992). The lower claudin-4 expression may be associated with proximal invasion via loss of the tight junction. In accordance with our results, Soini et al. also showed that claudin-4 expression is lower in diffuse-type gastric cancer (Soini et al., 2006). Recently, the expression of claudin-4 in non-neoplastic gastric mucosa especially that with intestinal metaplasia was reported by Matsuda et al. (2007). They showed that claudin-4 expression at the invasive front of gastric cancer and it may be a marker of intestinal differentiation.

Recent studies have indicated modulatory effects of claudins on MMP activation. To understand the mechanisms underlying claudin-induced increasing MMP activity, we tested the relationships between MMP-2, MMP-9 expression and claudin-4 expression. We found claudin-4 expression was significantly associated with MMP-9 expression, but not with MMP-2 expression. Whether there is a cause and effect relationship between MMP-9 and claudin-4 is not clear. Recently, Oku et al. showed that claudin-1 enhanced the invasive activity of oral squamous cell carcinoma cells by promoting cleavage of laminin-5 γ2 chain via MMP-2 and membrane-type MMP-1 (Oku et al., 2006). Agarwal et al. also showed that claudin-3 and claudin-4 expression in ovarian epithelial cells enhanced invasion and was associated with increased MMP-2 activity. The above data imply that claudins may regulate MMP

activity (Agarwal et al., 2005).

In conclusion, this study revealed that all of MMP-2, MMP-9 and claudin-4 expression were significantly higher in intestinal-type than in diffuse-type gastric cancer. The results implied that MMP-2, MMP-9 and claudin-4 expression may be phenotypic features distinguishing these two types of gastric cancer. The relationships between MMP-2 and MMP-9 expression with claudin-4 expression showed that claudin-4 expression was significantly associated with MMP-9 expression, but not with MMP-2 expression. Possibly, claudin-4 played a role in determining MMP-9 activity which favored intestinal-type gastric cancer to metastasize to liver (Umehara et al., 1992).

Acknowledgements. This study was supported by a grant NSC 95-2622-B-238-001-CC3 from the National Science Council and a grant CMRPG32050 from the Chang Gung Memorial Hospital, Taiwan.

References

Agarwal R., D'Souza T. and Morin P.J. (2005). Claudin-3 and claudin-4 expression in ovarian epithelial cells enhances invasion and is associated with increased matrix metalloproteinase-2 activity. Cancer Res. 65, 7378-7385.

Balda M.S., Garrett M.D. and Matter K. (2003). The ZO-1-associated Y-box factor ZONAB regulates epithelial cell proliferation and cell density. J. Cell Biol. 160, 423-432.

Boag A.H. and Young I.D. (1994). Increased expression of the 72-kd type IV collagenase in prostatic adenocarcinoma. Demonstration by immunohistochemistry and in situ hybridization. Am. J. Pathol. 144, 585-591.

Gonzalez-Mariscal L., Betanzos A. and Nava P. (2003). Tight junction proteins. Prog. Biophys. Mol. Biol. 81, 1-44.

Hart I.R. and Saini A. (1992). Biology of tumour metastasis. Lancet 339, 1453-1457.

Itoh M. and Bissell M.J. (2003). The organization of tight junctions in epithelia: implications for mammary gland biology and breast tumorigenesis. J. Mammary Gland Biol. Neoplasia 8, 449-462.

Itoh M., Furuse M., Morita K., Kubota K., Saitou M. and Tsukitas S. (1999). Direct binding of three tight junction-associated MAGUKs, ZO-1, ZO-2, and ZO-3, with the COOH termini of claudins. J. Cell Biol. 147, 1351–1363.

Kohn E.C. and Liotta L.A. (1995). Molecular insights into cancer invasion: strategies for prevention and intervention. Cancer. Res. 55, 1856-1862.

Kuo W.L., Lee L.Y., Wu C.M., Wang C.C., Yu J.S., Liang Y., Lo C.H., Huang K.H. and Hwang T.L. (2006). Differential expression of claudin-4 between intestinal and diffuse-type gastric cancer. Oncol. Rep. 16, 729-734.

Langbein L., Pape U.F., Grund G., Kuhn C., Praetzel S., Moll I., Moll R. and Franke W.W. (2003). Tight junction-related structures in the absence of a lumen: occludin, claudins and tight junction plaque proteins in densely packed cell formations of stratified epithelia and squamous cell carcinomas. Eur. J. Cell Biol. 82, 385-400.

Lauren P. (1965). The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type of carcinoma: An attempt at histochemical classification. Acta Pathol. Microbiol. Scand. 64, 31-49.

Martin T.A. and Jiang W.G. (2001). Tight junctions and their role in cancer metastasis. Histol. Histopathol. 16, 1183-1195.

Matsuda Y., Semba S., Ueda J., Fuku T., Hasuo T., Chiba H., Sawada N., Kuroda Y. and Yokozaki H. (2007). Gastric and intestinal claudin expression at the invasive front of gastric carcinoma. Cancer Sci. 98, 1014-1019.

Matter K. and Balda M.S. (2003). Signalling to and from tight junctions. Nat. Rev. Mol. Cell Biol. 4, 225-236.

Mitic L.L., Van Itallie C.M. and Anderson J.M. (2000). Molecular physiology and pathophysiology of tight junctions I. Tight junction structure and function: lessons from mutant animals and proteins. Am. J. Physiol. Gastrointest. Liver Physiol. 279, G250-254.

Morita K., Furuse M., Fujimoto K. and Tsukita S. (1999). Claudin multigene family encoding four-transmembrane domain protein components of tight junction strands. Proc. Natl. Acad. Sci. USA 96, 511-516.

Morson B.C., Dawson I.M.P. and Day D.W. (1990). Morson and Dawson's gastrointestinal pathology. Third ed. Blackwell Science. Oxford. pp 53-70.

Mrena J., Wiksten J.P. and Nordling S. (2006). MMP-2 but not MMP-9 associated with COX-2 and survival in gastric cancer. J. Clin. Pathol. 59, 618-623.

Mullin J.M. (2004). Epithelial barriers, compartmentation, and cancer. Sci. STKE 216, pe2.

Murphy G. and Docherty A.J. (1992) The matrix metalloproteinases and their inhibitors. Am. J. Respir. Cell. Mol. Biol. 7, 120-125.

Murray G.I., Duncan M.E., Arbuckle E., Melvin W.T. and Fothergill J.E. (1998). Matrix metalloproteinase and their inhibitors in gastric cancer. Gut 43, 791-797.

Oku N., Sasabe E., Ueta E., Yamamoto T. and Osaki T. (2006). Tight junction protein claudin-1 enhances the invasive activity of oral squamous cell carcinoma cells by promoting cleavage of laminin-5 gamma2 chain via matrix metalloproteinase (MMP)-2 and membrane-type MMP-1. Cancer Res. 66, 5251-5257..

Ravn V., Rasmussen B.B. and Hojholt L. (1993). Reproducibility of subjective immunohistochemical estrogen- and progesterone-receptor determination in human endometrium. Pathol. Res. Pract. 189, 1015-1022.

Ravn V., Havsteen H. and Thorpe S.M. (1998). Immunohistochemical evaluation of estrogen and progesterone receptors in paraffin-embedded, formalin-fixed endometrial tissues: comparison with enzyme immunoassay and immunohistochemical analysis of frozen tissue. Mod. Pathol. 11, 709-715.

Sakurai Y., Otani Y., Kameyama K., Hosoda Y., Okazaki I., Kubota T., Kumai K. and Kitajima M. (1997). Expression of interstitial collagenase (matrix metalloproteinase-1) in gastric cancers. Jap. J. Cancer Res. 88, 401-406.

Schraml P., Bucher C., Bissig H., Nocito A., Haas P., Wilber K., Seelig S., Kononen J., Mihatsch M.J., Dirnhofer S. and Sauter G.. (2003). Cyclin E overexpression and amplification in human tumours. J. Pathol. 200, 375-382.

Soini Y., Tommola S., Helin H. and Martikainen P. (2006). Claudins 1, 3, 4 and 5 in gastric carcinoma, loss of claudin expression associates with the diffuse subtype. Virchows Arch. 448, 52-58.

Sugano H., Nakamura K. and Kato Y. (1982). Pathological studies of human gastric cancer. Acta Pathol. Jpn. 32, 329-347.

Torii A., Kodera Y., Uesaka K., Hirai T., Yasui K., Morimoto T., Yamamura Y., Kato T., Hayakawa T., Fujimoto N. and Kito T.

(1997). Plasma concentration of matrix metalloproteinase 9 in gastric cancer. Br. J. Surg. 84, 133-136.

Tsukita S., Furuse M. and Itoh M. (2001). Multifunctional strands in tight junctions. Nat. Rev. Mol. Cell Biol. 2, 285-293.

Umehara Y., Kimura T., Yoshida M., Oba N. and Harada Y. (1992). Metastatic mode of gastric carcinoma by flow cytometric and clinicopathologic parameters. Clin. Exp. Metastasis 10, 19-24.

Urbanski S.J., Edwards D.R., Hershfield N., Huchcroft S.A., Shaffer E., Sutherland L. and Kossakowska A.E. (1993). Expression pattern of metalloproteinases and their inhibitors changes with the progression of human sporadic colorectal neoplasia. Diagn. Mol. Pathol. 2, 81-89.

Wu C.M., Lee Y.S., Wang T.H., Lee L.Y., Kong W.H., Chen E.S., Wei M.L., Liang Y. and Hwang T.L. (2006) Identification of differential gene expression between intestinal and diffuse gastric cancer using cDNA microarray. Oncol Rep 15: 57-64, 2006.

Wu Y., Dowbenko D., Spencer S., Laura R., Lee J., Gu Q. and Lasky L.A. (2000). Interaction of the tumor suppressor PTEN/MMAC with a PDZ domain of MAGI3, a novel membrane-associated guanylate kinase. J. Biol. Chem. 275, 21477–21485.

Yamamoto Y., Nishimura N., Morimoto S., Kitamura H., Manabe S., Kanayama H.O., Kagawa S. and Sasaki T. (2003). Distinct roles of Rab3B and Rab13 in the polarized transport of apical, basolateral, and tight junctional membrane proteins to the plasma membrane. Biochem. Biophys. Res. Commun. 308, 270–275.

Accepted October 24, 2007