The Clinicopathological and Prognostic Significance of MUC-1 Expression in Japanese Gastric Carcinomas: An Immunohistochemical Study of Tissue Microarrays

XIAO-HAN LI[1,3*], HUA-CHUAN ZHENG[2*], ZHI-GANG WANG[2], HIROYUKI TAKAHASHI[1], XIANG-HONG YANG[3], YI-FU GUAN[2] and YASUO TAKANO[1]

[1]*Department of Diagnostic Pathology, Graduate School of Medicine and Pharmaceutical Science, University of Toyama, Sugitani 2630, Toyama, Japan;*
[2]*Department of Biochemistry and Molecular Biology, College of Basic Medicine, China Medical University, Shenyang;*
[3]*Division of Pathology, Shengjing Hospital of China Medical University, Shenyang, China*

Abstract. *Background: MUC-1 is synthesized as a single polypeptide that then undergoes proteolytic cleavage, and is associated with the epidermal growth factor receptor tyrosine kinases. In malignancies, MUC-1 may function as an anti-adhesion molecule, but can also promote adhesion and presumably metastasis. Materials and Methods: Expression of MUC-1, -2, -4 and -5AC was evaluated on tissue microarrays of gastric carcinomas (n=237) and adjacent non-cancerous mucosa specimens (n=89) by immunohistochemistry and compared with clinicopathological parameters and survival time of the patients. Results: MUC-1 was found to be highly expressed in gastric carcinomas in comparison with non-cancerous mucosa ($p<0.05$) and positively correlated with depth of invasion, lymphatic and venous invasion, lymph node metastasis, UICC staging and MUC-4 expression ($p<0.05$), but not with age, tumor size, MUC-2 or MUC-5AC expression ($p>0.05$). Intestinal-type carcinomas showed more MUC-1 expression than their diffuse-type counterparts ($p<0.05$). Kaplan-Meier analysis indicated that the cumulative survival rate of patients with no MUC-1 expression was significantly higher than those with weak, moderate or strong expression in gastric carcinomas ($p<0.05$), but no difference was observed when tumors were stratified according to the depth of invasion ($p>0.05$). Cox's analysis showed three independent prognostic factors, depth of invasion, lymphatic invasion and venous invasion, to affect the relationship between MUC-1 expression and prognosis. Conclusion: Up-regulation of MUC-1 expression may be involved in pathogenesis, invasion, metastasis and differentiation of gastric carcinoma. Altered expression might therefore be employed as an indicator of pathobiological behavior of gastric carcinoma. MUC-1 expression was found to be a prognostic factor for gastric carcinoma patients, albeit not independent of parameters of invasion.*

Despite a worldwide decline in incidence and mortality since the second half of the 20th century, gastric cancer still ranks as the fourth most common cancer and the second most frequent cause of death from cancer, accounting for 10.4% of cancer deaths worldwide. It continues to be a major health concern because of the slow decrease in incidence in Asia and high mortality from diagnosed gastric carcinomas in the West (1, 2). Malignant transformation of gastric epithelial cells is biologically a multi-step and multi-factorial process, resulting from accumulated genetic alterations. When the stomach suffers from infection with *Helicobacter pylori* (HP), a group I carcinogen, HP lipopolysaccharides decrease the synthesis of mucins, which normally form a gel layer and function as a protective and lubricating factor against luminal acid, proteolytic enzymes and DNA damage from various carcinogens (3, 4).

Mucins are high molecular weight epithelial glycoproteins that are heavily glycosylated with many oligosaccharide side chains linked to a protein backbone called apomucin (5). Thus far, at least 19 mucins have been identified and divided into two distinct classes according to their structure and function: (i) secreted types: MUC-2, -5AC, -5B, -6, -7, -8, -9 and -19; (ii) membrane-associated types: MUC-1, -3A, -3B, -4, -12, -13, -15, -16, -17 and -20 (6). Secreted mucins are glycoproteins constituting the major macromolecular

*These authors contributed equally to this work.

Correspondence to: Professor Hua-chuan Zheng, Department of Biochemistry and Molecular Biology, College of Basic Medicine, China Medical University, Shenyang, China. e-mail: zheng_huachuan@hotmail.com

Key Words: Gastric carcinoma, MUC-1, pathobiological behavior, prognosis.

component of mucus, while membrane-associated mucins contribute to epithelial cell–cell interactions (7). Their patterns of expression, especially of secreted mucins, appear to be relatively cell-, tissue-, or organ- specific (6). Qualitative and quantitative alteration of mucin expression in preneoplastic and neoplastic lesions has suggested potential roles in neoplastic processes, as reviewed by Cozzi *et al*. (7). Furthermore, numerous pieces of evidence indicate a close association between aberrant mucin expression and aggressive behavior of malignancies (6-12).

MUC-1 is synthesized as a single polypeptide that then undergoes proteolytic cleavage, creating a heterodimer that remains associated during its post-translational processing in the Golgi apparatus (13). MUC-1 protein belongs to the group of membrane-bound mucins that contain a large extracellular domain with 30-90 tandem repeats of the 20-amino acid peptide PAPGSTAPPAHGVTSAPDTR, along with a 31-amino acid transmembrane domain and a short cytoplasmic tail (11). The MUC-1 gene can generate isoforms MUC-1/TM, MUC-1/Y, MUC1-/Z and MUC-1/SEC by alterative splicing (14). MUC-1 has been shown to be associated with the epidermal growth factor receptor tyrosine kinases through the binding of its cytoplasmic tail to Grb2/SOS, indicating an involvement in cellular signaling (15). In malignancies, MUC-1 may function as an anti-adhesion molecule facilitating release of cells from tumor nests (8, 11,16). It can paradoxically also promote adhesion and presumably metastasis by presenting carbohydrate ligands to adhesion molecules on endothelial cells (17). It has further been proposed that enhanced levels of MUC1 expression on cancer cells may mask extracellular domains from immune surveillance, confer a survival advantage and play an important role in the ability of tumors to invade and metastasize (7, 16).

Japan is a high-risk area of gastric carcinoma worldwide and the observed gastric carcinomas are characterized as follows: (i) predominance in the distal stomach; (ii) frequently detected at an early stage (nearly 50%); (iii) mostly restricted to the elderly population; (iv) comparatively good prognosis (1). In this study, the expression of MUC-1, 2, 4 and 5AC was investigated with intermittent microwave immunohistochemistry and tissue microarrays (TMAs) from 237 Japanese gastric carcinomas to clarify clinicopathological and prognostic roles of MUC-1 expression.

Materials and Methods

Patients. Gastric carcinomas (n=237) and adjacent non-cancerous mucosa (n=89) were collected from surgical resection at our related hospital from 1993 to 2002. The patients with gastric carcinomas were 170 men and 67 women (35-88 years, mean=66.2years). Nodal metastasis or involvement was detected in 95 cases. None of the patients underwent chemotherapy or radiotherapy before surgery. All provided consent for use of tumor tissue for clinical research and our University Ethical Committee approved the research protocol. All patients were followed up by consulting their case documents and by telephone.

Pathology. All tissues were fixed in 10% neutralized formalin, embedded in paraffin and cut into 4 μm sections stained with hematoxylin and eosin (HE) to confirm the histological diagnosis and microscopic characteristics. The staging for each gastric carcinoma was evaluated according to the Internationale le Contre Cancer (UICC) system indicating the extent of tumor spread (18). Histological architecture was defined in terms of Lauren's classification (19). Furthermore, tumor size, depth of invasion, lymphatic and venous invasion, and lymph node metastasis of tumors were determined.

Tissue microarray (TMA). From HE stained sections of the selected tumor cases, representative areas of solid tumor were selected for sampling and 2 mm-diameter tissue cores per donor block were punched out and transferred to a recipient block with a maximum of 48 cores using a Tissue Microarrayer (AZUMAYA KIN-1, Japan). Thin sections (4 μm) were consecutively cut from the microarrays and transferred to polylysine-coated glass slides. HE staining was performed for confirmation of tumor tissue (Figure 1a).

Immunohistochemistry. Serial sections of TMA were deparaffinized with xylene, dehydrated with alcohol, and subjected to immunohistochemical staining with intermittent microwave radiation as previously described (20). Mouse anti-human MUC-1 (NovoCastra,UK), anti-human MUC-2 (NovoCastra), anti-human MUC-4 (NovoCastra) and anti-human MUC-5AC (NovoCastra) antibodies were used at 1: 100 dilution to detect the respective proteins, with anti-mouse Envison-PO (DAKO, USA) as the secondary antibody. Binding was visualized with 3,3'-diaminobenzidine (DAB) and counterstaining with Mayer's hematoxylin was performed to aid orientation. Omission of the primary antibody was used as a negative control.

Immunoreactivity for MUC-1 and MUC-4 was localized in the cytoplasm and membrane, while MUC-2 and MUC-5AC showed a cytoplasmic pattern (Figure 1 b-i). One hundred cells were randomly selected and counted from five representative fields of each section blindly by three independent observers (Takano Y, Zheng HC and Li XH) and the percentages of positive cells in the total counted were graded semi-quantitatively using a four-tier scoring system: negative (–), 0–5%; weakly positive (+), 6–25%; moderately positive (++), 26–50%; and strongly positive (+++), 51–100%.

Statistical analysis. Statistical evaluation was performed using the Spearman correlation test to analyze rank data. Kaplan–Meier survival plots were generated and comparisons between survival curves were made with the log-rank statistic. The Cox proportional hazards model was employed for multivariate analysis. SPSS 10.0 software was applied to analyze all data and $p<0.05$ was considered statistically significant.

Results

As indicated in Figure 1, MUC-1 was detected in some gastric glands, but not in the surface epithelium, intestinal metaplasia or gastric pits. However, MUC-2 was strongly

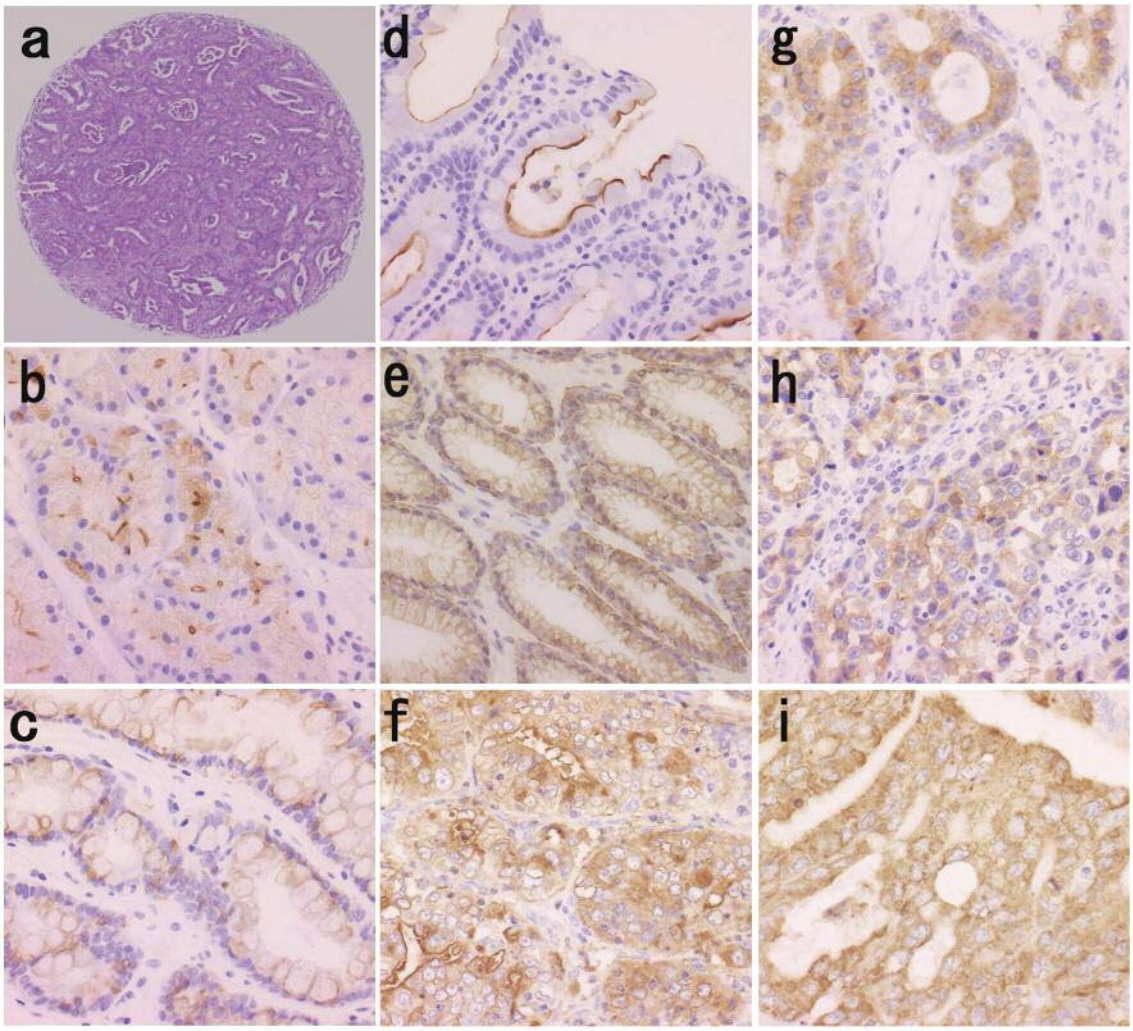

Figure 1. *HE staining and immunohistochemistry of gastric samples. HE staining of TMA of gastric carcinoma (a). Immunoreactivity to MUC-1 and MUC-4 was distributed to the cytoplasm and membrane, while MUC-2 and MUC-5AC staining was limited to the cytoplasm. MUC-1 was detected in some gastric glands (b), while MUC-2 was positively expressed in goblet cells of intestinal metaplasia (c). MUC-4(d) and MUC-5AC (e) were immunoreactive mainly in the surface epithelial cells. There was strong diffuse expression of MUC-1(f), MUC-2(g), MUC-4(h) and MUC-5AC in gastric carcinoma (i).*

expressed in goblet cells of intestinal metaplasia. MUC-4 was immunoreactive mainly in the membranes of the surface epithelial cells and gastric pits, and occasionally in vascular endothelial cells. MUC-5AC positivity was observed in superficial epithelium, gastric pits and some gastric glands. There was strong diffuse expression of MUC-1, -2, -4 and -5AC in the cytoplasm of carcinoma (Figure 1). Statistically, MUC-1 expression was greater in gastric carcinomas than in adjacent non-cancerous mucosa ($p<0.05$, Table I).

Table I. *MUC-1 expression in gastric carcinomas.*

Groups	n	MUC-1 expression					
		–	+	++	+++	PR(%)	*P*-value
Non-cancerous mucosa	89	68	19	2	0	23.5	<0.001
Carcinomas	237	132	46	22	37	44.3	

PR: positive rate.

As summarized in Table II, MUC-1 expression was positively correlated with the depth of invasion, lymphatic and venous invasion, lymph node metastasis, UICC staging and MUC-4 expression ($p<0.05$). There was significant variation in MUC-1 expression with age, tumor size, and MUC-2 or -5AC expression ($p>0.05$). Intestinal-type carcinomas showed more MUC-1 expression than their diffuse-type counterparts ($p<0.05$).

Table II. *Relationship between MUC-1 expression and clinicopathological features of gastric carcinomas.*

Clinico-pathological feature	n	MUC-1 expression					
		–	+	++	+++	PR(%)	*P*-value
Age (years)							0.256
<65	92	57	14	5	16	38.0	
≥65	145	75	32	17	21	48.3	
Tumor size (cm)							0.101
<4	115	68	25	11	11	40.9	
≥4	122	64	21	11	26	47.5	
Depth of invasion							0.007
Tis-T1	120	75	25	9	11	37.5	
T2-T4	117	57	21	13	26	51.3	
Lymphatic invasion							0.005
–	147	91	29	11	16	38.1	
+	90	41	17	11	21	54.4	
Venous invasion							<0.001
–	210	124	45	18	23	41.0	
+	27	8	1	4	14	70.4	
Lymph node metastasis							0.011
–	142	87	27	13	15	38.7	
+	95	45	19	9	22	52.6	
UICC staging							0.007
0-I	147	90	29	13	15	38.8	
II-IV	90	42	17	9	22	53.3	
Lauren's classification							0.002
Intestinal type	131	63	26	15	27	51.9	
Diffuse type	106	69	20	7	10	34.9	
MUC-2 expression							0.475
–	156	85	29	16	26	45.5	
+	81	47	17	6	11	42.0	
MUC-4 expression							0.004
–	127	81	22	10	14	36.2	
+	110	51	24	12	23	53.6	
MUC-5AC expression							0.233
–	99	51	19	12	17	48.5	
+	138	81	27	10	20	41.3	

PR, positive rate; T_{is}: carcinoma *in situ*; T_1: involvement of the lamina propria and submucosa; T_2: muscularis propria and subserosa; T_3: serosa; T_4: invasion through the serosa.

Follow-up information was available on 237 of the gastric carcinoma patients for periods ranging from 0.2 months to 12.2 years (mean=70.8 months). Figure 2 shows survival curves stratified according to MUC-1 expression for all (a), early(b) and advanced (c) gastric carcinomas. Univariate analyses using the Kaplan-Meier method indicated cumulative survival rate of patients with no MUC-1 expression to be significantly higher than these patients with weak, moderate or strong expression in all gastric carcinomas ($p<0.05$), but no difference was observed when tumors were stratified according to the depth of invasion ($p>0.05$). Multivariate analysis using the Cox's proportional

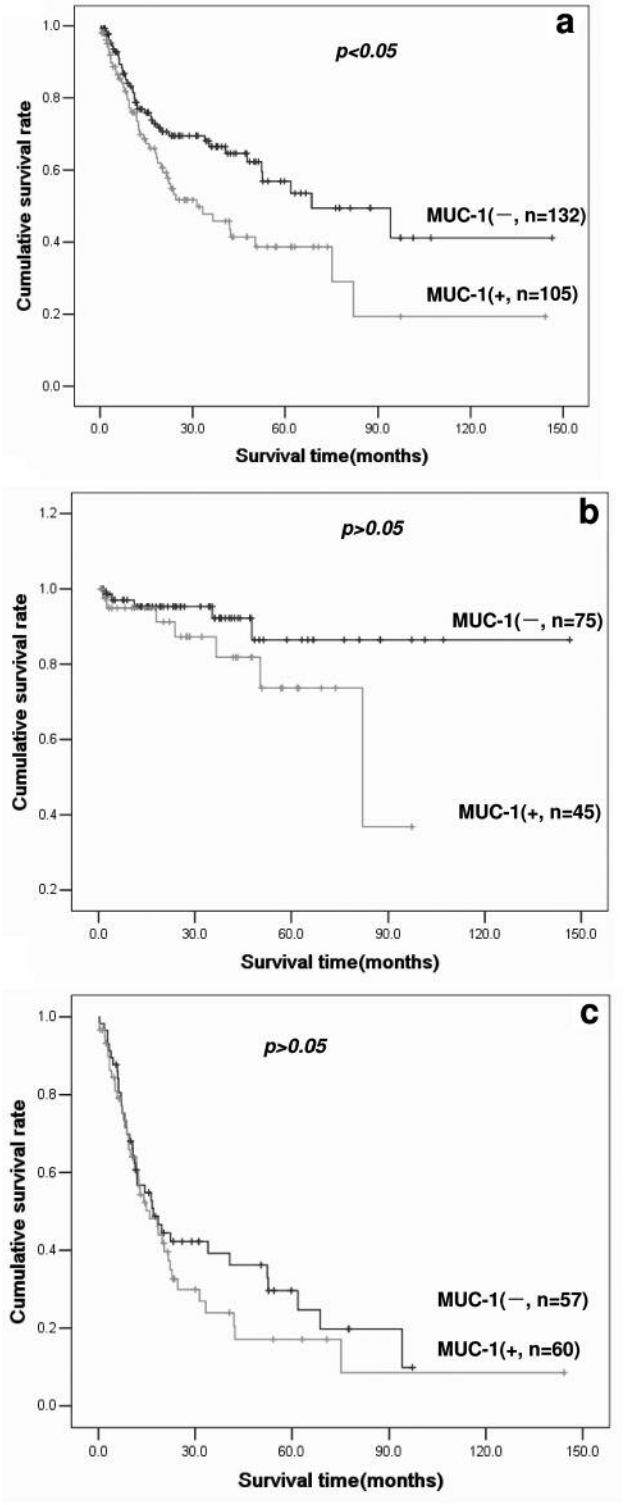

Figure 2. *Correlation between Status of MUC-1 and prognosis of the gastric carcinoma patients. Kaplan-Meier curves for cumulative survival rates of patients with all (a), early (EGC, b) and advanced (AGC, c) gastric carcinomas according to MUC-1 expression. EGC, invasion within the mucosa and submucosa; AGC, invasion below muscularis properia.*

Table III. *Multivariate analysis of clinicopathological variables for survival with gastric carcinomas.*

Group	Clinicopathological parameter	Relative risk (95% CI)	*P*-value
A	Age (≥65 years)	2.175(1.351-3.501)	0.001
B	Tumor size (≥4 cm)	1.613(0.849-3.064)	0.144
C	Depth of invasion (into muscularis propria)	5.176(2.074-12.919)	<0.001
D	Lymphatic invasion (+)	4.402(2.477-7.824)	<0.001
E	Venous invasion (+)	1.856(1.115-3.089)	0.017
F	Lymph node metastasis (+)	2.376(0.748-7.544)	0.142
G	UICC staging (II-IV)	0.374(0.116-1.203)	0.099
H	Lauren's classification (Diffuse-type)	1.324(0.797-2.200)	0.279
I	MUC-1 expression (+ to +++)	1.297(0.824-2.040)	0.261

CI: confidence interval.

hazard model indicated that age, depth of invasion, lymphatic and venous invasion, but not MUC-1, were independent prognostic factors (Table III). Further analysis showed that these three local invasion factors influenced the relationship between MUC-1 expression and survival time of carcinoma patients (Table IV).

Table IV. *Multivariate analysis of MUC-1 expression and other concordant factors for survival with gastric carcinomas.*

Groups(from Table III)	H Relative risk (95% CI)	*P*-value
I	1.662(1.100-2.512)	0.016
I+C	1.387(0.916-2.101)	0.122
I+D	1.257(0.826-1.914)	0.286
I+E	1.396(0.910-2.141)	0.126
I+C+D	1.208(0.796-1.833)	0.375
I+C+E	1.282(0.840-1.957)	0.250
I+D+E	1.094(0.708-1.691)	0.686
I+C+D+E	1.096(0.714-1.682)	0.676

CI: confidence interval.

Discussion

In the present study, we found a low positive rate for MUC-1 in gastric mucosa, lower than in gastric carcinomas, in line with previous reports (9, 11). Thus MUC-1 up-regulation may play some role in the malignant transformation of gastric epithelial cells. Enhanced MUC-1 expression has been observed in several other kinds of malignancies, such as breast, colon and pancreatic cancer (10, 12, 16). Increased expression of MUC-1 is generally thought to be related to the level of transcription or gene dosage (21, 22). An increase of MUC-1 mRNA expression after stimulation of cultured gastric carcinoma cells with TNF-α has already been observed (23) and IFN-γ and TNF-α have synergistic effects on the cell surface expression of MUC-1 in normal breast epithelium and breast cancer cells (24). Therefore, these cytokines in gastric carcinomas or the tumor microenvironment might be postulated to induce *MUC-1* mRNA expression and subsequently up-regulate its protein level.

Changed MUC-1 expression in cancer progression and metastasis is characterized by increased levels, altered glycosylation, and aberrant surface distribution patterns (7). In the present study, the results demonstrated gastric carcinomas with positive MUC-1 expression to display deep invasion, frequent lymphatic or venous invasion, more lymph node metastasis and high tumor staging, in line with earlier findings (8, 9, 11) indicating that up-regulated MUC-1 expression is closely linked to progression and might be considered as a good indicator of pathobiological behavior. Indeed, Suwa *et al*. (25) found that MUC-1 cDNA transfection into human gastric cancer cells enhanced *in vivo* growth and invasiveness. The MUC-1 cytoplasmic domain might compete for and interact with β-catenin through a similar motif as E-cadherin, thus suppressing cell–cell adhesion (26).

Previous reports provided evidence that mucin expression is also closely associated with differentiation of gastric carcinomas (6, 27). In the present study, it was found that intestinal-type gastric carcinomas had higher MUC-1 expression compared with diffuse counterpart lesions. Baldus's data are also consistent with our findings using both monoclonal and polyclonal antibodies against MUC-1 (27). Therefore, we hypothesized that MUC-1 is a possible differentiation indicator of gastric carcinoma and could be employed to separate intestinal- from diffuse-type carcinomas. It should be noted that Wang *et al*. and Kocer *et al*. reported no difference in MUC-1 expression between the two types of carcinoma (8, 28). The discrepancy might be attributable to their small numbers of cancer cases or population differences. Asia is epidemiologically a high-incidence area of gastric carcinoma, which determined the similar MUC-1 expression pattern in Japan, Taiwan and Chongqing of China (8, 11).

Regarding our experimental methods, we used microwave intermittent irradiation for incubation of primary and secondary antibodies, which is known to increase sensitivity and specificity of immunohistochemistry. For statistics, the Spearman correlation analysis was employed for the present rank data, making full use of our semi-quantitative results. Additionally, the specimens used in this study were fixed in freshly-prepared 10% formalin for periods of up to 4 days, which facilitates antigen retrieval. These three aspects improved our data's reliability in combination with a large number of Japanese carcinomas.

To clarify the prognostic significance, here we analyzed the relation of MUC-1 expression with the survival of 237 patients with gastric carcinoma and revealed a link between its positivity and poor survival, in concordance with previous reports (8, 27-29). If stratified according to invasion of depth, this close association disappears, indicating that MUC-1 was not an independent factor for the prognosis of gastric carcinoma patients in the present study. Multivariate analysis demonstrated three independent prognostic factors, depth of invasion, lymphatic and venous invasion as influencing the relationship between MUC-1 expression and prognosis, revealing that the prognostic significance of MUC-1 is dependent on local invasion in gastric carcinoma. However, it was documented that MUC-1 could be employed as an independent factor for worse prognosis of gastric carcinoma using PEM, but not HMFG-2 antibody, providing an evidence that the prognostic significance of MUC-1 was obtained in some specific conditions.

In summary, up-regulation of MUC-1 expression may be involved in pathogenesis, invasion and metastasis of gastric carcinomas. MUC-1 might underlie the molecular mechanisms for the differentiation of intestinal- and diffuse-type carcinomas. Altered expression might therefore be employed as an indicator of pathobiological behavior and prognosis of gastric carcinoma.

Acknowledgements

We particularly thank Tokimasa Kumada and Hideki Hatta for their technical help and Yukari Inoue for her secretarial assistance. This work was partially supported by the Japanese Ministry of Education, Science, Sports and Culture, Grant-in-Aid for Scientific Research 14770072, Japanese Smoking Society Foundation, Shenyang Outstanding Talent Foundation of China, and Natural Scientific Foundation of China (30600286).

References

1 Inoue M and Tsugane S: Epidemiology of gastric cancer in Japan. Postgrad Med J *81*: 419-424, 2005.

2 Zheng HC, Takahashi H, Murai Y, Cui ZG, Nomoto K, Miwa S, Tsuneyama K and Takano Y: Up-regulated EMMPRIN/CD147 might contribute to growth and angiogenesis of gastric carcinoma: a good marker for local invasion and prognosis. Br J Cancer *95*: 1371-1378, 2006.

3 Slomiany BL and Slomiany A: Impedance of *Helicobacter pylori* lipopolysaccharide interference with gastric mucin synthesis by peroxisome proliferator-activated receptor gamma activation involves phosphatidylinositol 3-kinase/ERK pathway. IUBMB Life *55*: 97-102, 2003.

4 Slomiany BL and Slomiany A: Up-regulation in endothelin-1 by *Helicobacter pylori* lipopolysaccharide interferes with gastric mucin synthesis via epidermal growth factor receptor transactivation. Scand J Gastroenterol *40*: 921-928, 2005.

5 Hollingsworth MA and Swanson BJ: Mucins in cancer: protection and control of the cell surface. Nat Rev Cancer *4*: 45-60, 2004.

6 Zheng H, Takahashi H, Nakajima T, Murai Y, Cui Z, Nomoto K, Tsuneyama K and Takano Y: MUC6 down-regulation correlates with gastric carcinoma progression and a poor prognosis: an immunohistochemical study with tissue microarrays. J Cancer Res Clin Oncol *132*: 817-823, 2006.

7 Cozzi PJ, Wang J, Delprado W, Perkins AC, Allen BJ, Russell PJ and Li Y: MUC1, MUC2, MUC4, MUC5AC and MUC6 expression in the progression of prostate cancer. Clin Exp Metastasis *22*: 565-573, 2005.

8 Wang RQ and Fang DC: Alterations of MUC1 and MUC3 expression in gastric carcinoma: relevance to patient clinicopathological features. J Clin Pathol *56*: 378-384, 2003.

9 Akyürek N, Akyol G, Dursun A, Yamaç D and Günel N: Expression of MUC1 and MUC2 mucins in gastric carcinomas: their relationship with clinicopathologic parameters and prognosis. Pathol Res Pract *198*: 665-674, 2002.

10 Huang WB, Shi LH, Zhu XQ, Zhu XQ, Xu GX and Qi Q: Expression of mucin MUC1 and MUC2 in colorectal carcinoma and their clinical significance. Ai Zheng *21*: 1231-1234, 2002.

11 Wang JY, Chang CT, Hsieh JS, Lee LW, Huang TJ, Chai CY and Lin SR: Role of MUC1 and MUC5AC expressions as prognostic indicators in gastric carcinomas. J Surg Oncol *83*: 253-260, 2003.

12 Levi E, Klimstra DS, Andea A, Basturk O and Adsay NV: MUC1 and MUC2 in pancreatic neoplasia. J Clin Pathol *57*: 456-462, 2004.

13 Fattorossi A, Battaglia A, Malinconico P, Stoler A, Andreocci L, Parente D, Coscarella A, Maggiano N, Perillo A, Pierelli L and Scambia G: Constitutive and inducible expression of the epithelial antigen MUC1 (CD227) in human T cells. Exp Cell Res *280*: 107-118, 2002.

14 Wreschner DH, McGuckin MA, Williams SJ, Baruch A, Yoeli M, Ziv R, Okun L, Zaretsky J, Smorodinsky N, Keydar I, Neophytou P, Stacey M, Lin HH and Gordon S: Generation of ligand-receptor alliances by "SEA" module-mediated cleavage of membrane-associated mucin proteins. Protein Sci *11*: 698-706, 2002.

15 Pandey P, Kharbanda S and Kufe D: Association of the DF3/MUC1 breast cancer antigen with Grb2 and the Sos/Ras exchange protein. Cancer Res *55*: 4000-4003, 1995.

16 Rakha EA, Boyce RW, Abd El-Rehim D, Kurien T, Green AR, Paish EC, Robertson JF and Ellis IO: Expression of mucins (MUC1, MUC2, MUC3, MUC4, MUC5AC and MUC6) and their prognostic significance in human breast cancer. Mod Pathol *18*: 1295-1304, 2005.

17 Yu LG, Andrews N, Zhao Q, McKean D, Williams JF, Connor LJ, Gerasimenko OV, Hilkens J, Hirabayashi J, Kasai K and Rhodes JM: Galectin-3 interaction with Thomsen-Friedenreich disaccharide on cancer-associated MUC1 causes increased cancer cell endothelial adhesion. J Biol Chem *282*: 773-781, 2007.

18 Sobin LH and Wittekind CH: TNM Classification of malignant tumors, 6th edn. New Jersey: John Wiley & Sons, Hoboken, 2002.
19 Zheng H, Takahashi H, Murai Y, Cui Z, Nomoto K, Miwa S, Tsuneyama K and Takano Y: Pathobiological characteristics of intestinal and diffuse-type gastric carcinoma in Japan: an immunostaining study on the tissue microarray. J Clin Pathol *60*: 273-277, 2007.
20 Kumada T, Tsuneyama K, Hatta H, Ishizawa S and Takano Y: Improved 1-h rapid immunostaining method using intermittent microwave irradiation: practicability based on 5 years application in Toyama Medical and Pharmaceutical University Hospital. Mod Pathol *17*: 1141-1149, 2004.
21 Gaemers IC, Vos HL, Volders HH, van der Valk SW and Hilkens J: A stat-responsive element in the promoter of the episialin/MUC1 gene is involved in its overexpression in carcinoma cells. J Biol Chem *276*: 6191-6199, 2001.
22 Bieche I and Lidereau R: A gene dosage effect is responsible for high overexpression of the MUC1 gene observed in human breast tumors. Cancer Genet Cytogenet *98*: 75-80, 1997.
23 Grohmann GP, Schirmacher P, Manzke O, Hanisch FG, Dienes HP and Baldus SE: Modulation of MUC1 and blood group antigen expression in gastric adenocarcinoma cells by cytokines. Cytokine *23*: 86-93, 2003.
24 Lagow EL and Carson DD: Synergistic stimulation of MUC1 expression in normal breast epithelia and breast cancer cells by interferon-gamma and tumor necrosis factor-alpha. J Cell Biochem *86*: 759-772, 2002.
25 Suwa T, Hinoda Y, Makiguchi Y, Takahashi T, Itoh F, Adachi M, Hareyama M and Imai K: Increased invasiveness of MUC1 and cDNA-transfected human gastric cancer MKN74 cells. Int J Cancer *76*: 377-382, 1998.
26 Yamamoto M, Bharti A, Li Y and Kufe D: Interaction of the DF3/MUC1 breast carcinoma- associated antigen and beta-catenin in cell adhesion. J Biol Chem *272*: 12492-12494, 1997.
27 Baldus SE, Zirbes TK, Engel S, Hanisch FG, Mönig SP, Lorenzen J, Glossmann J, Fromm S, Thiele J, Pichlmaier H and Dienes HP: Correlation of the immunohistochemical reactivity of mucin peptide cores MUC1 and MUC2 with the histopathological subtype and prognosis of gastric carcinomas. Int J Cancer *79*: 133-138, 1998.
28 Kocer B, Soran A, Kiyak G, Erdogan S, Eroglu A, Bozkurt B, Solak C and Cengiz O: Prognostic significance of mucin expression in gastric carcinoma. Dig Dis Sci *49*: 954-964, 2004.
29 Lee HS, Lee HK, Kim HS, Yang HK, Kim YI and Kim WH: MUC1, MUC2, MUC5AC, and MUC6 expressions in gastric carcinomas: their roles as prognostic indicators. Cancer *92*: 1427-1434, 2001.

Received November 13, 2007
Revised January 3, 2008
Accepted February 4, 2008