RESEARCH ARTICLE

Clinicopathological and Prognostic Significance of MUC-2, MUC-4 and MUC-5AC Expression in Japanese Gastric Carcinomas

Li-Jun Xiao[1,2], Shuang Zhao[1,2], En-Hong Zhao[3], Xin Zheng[1,2], Wen-Feng Gou[1], Ya-Nan Xing[4], Yasuo Takano[5], Hua-Chuan Zheng[1]*

Abstract

Background: The mucin components of the gastric gel layer function as a protective and lubricating factor against luminal acid and proteolytic enzymes. Alteration of mucin expression in gastric preneoplastic and neoplastic lesions has suggested potential roles in neoplastic processes. This study aimed to assess the clinicopathological and prognostic significance of MUC-2, MUC-4 and MUC-5AC in Japanese gastric cancer. Methods: Expression of MUC-2, -4 and -5AC was evaluated on tissue microarrays of gastric carcinomas and adjacent non-cancerous mucosa specimens by immunohistochemistry and compared with clinicopathological parameters and survival time of the patients. Results: The three mucins were found to be expressed to a lesser extent in gastric carcinomas in comparison with non-cancerous mucosa ($p<0.05$). MUC-2 expression was negatively correlated with tumor size, depth of invasion, and TNM staging of gastric cancer ($p<0.05$), while that of MUC-5AC was negatively associated with the depth of invasion, venous invasion, lymph node metastasis and TNM staging ($p<0.05$), but positively with MUC-4 and MUC-2 expression ($p<0.05$). There was higher MUC-2 expression in intestinal- than diffuse-type carcinomas ($p<0.05$). Kaplan-Meier analysis indicated no relationship between expression of the three mucins and the cumulative survival rate of patients, even stratified according to the depth of invasion ($p>0.05$). Conclusion: Down-regulated expression of MUC-2, -4 and -5AC may be involved in pathogenesis, invasion, metastasis or differentiation of gastric carcinoma. Their altered expression might therefore be employed as an indicator of pathobiological behavior.

Keywords: Gastric carcinoma - mucins-clinicopathological behaviors - prognosis - tumorigenesis

Asian Pacific J Cancer Prev, **13 (12)**, 6447-6453

Introduction

Despite a worldwide decline in incidence and mortality in the last 60 years, gastric cancer is still ranked as the fourth most common and the second most frequent cause of death from cancer. 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, even though advanced diagnostic and operative techniques are widely applied in clinical practice (Kelley et al., 2003; Rivera et al., 2007). Increased understanding of the changes that occur in gene expression in gastric cancer, particularly identification of novel biomarkers for cancer diagnosis and novel targets for treatment, may result in the improvement of diagnosis, treatment and prevention.

Mucins are a family of high molecular weight, heavily glycosylated proteins (glycoconjugates) with many oligosaccharide side chains linked to a protein backbone called apomucin. Their key function is their ability to form gels and act as a chemical barrier. Additionally, some mucins are associated with controlling mineralization, including nacre formation in molluscs, calcification in echinoderms and bone formation in vertebrates (McGuckin et al., 2011). They bind to pathogens as part of the immune system. 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 (Zheng et al., 2006; Li et al., 2008). Secreted mucins are glycoproteins constituting the major macromolecular component of mucus, while membrane-associated mucins contribute to epithelial cell-cell interactions. Their patterns

*[1]Department of Biochemistry and Molecular Biology, Institute of Pathology and Pathophysiology, College of Basic Medicine, China Medical University, [4]Department of Surgical Oncology, The First Affiliated Hospital of China Medical University, Shenyang, [2]Department of Immunology, Chengde Medical College, [3]Department of Surgical Oncology, the First Affiliated Hospital of Chengde Medical College,Chengde,China, [5]Clinical Research Institute, Kanagawa Cancer Center, Yokohama, Japan *For correspondence: zheng_huachuan@hotmail.com*

of expression, especially of secreted mucins, appear to be relatively cell-, tissue-, or organ- specific (Zheng et al., 2006). 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. (2005). Furthermore, numerous pieces of evidence indicate a close association between aberrant mucin expression and aggressive behaviors of malignancies (Akyürek et al., 2002; Huang et al., 2002; Wang et al., 2003; Wang et al., 2003; Levi et al., 2004; Cozzi et al., 2005; Zheng et al., 2006).

In the stomach, the mucin component of gastric gel layer functions as a protective and lubricating factor against luminal acid and proteolytic enzymes, which also hinders access of carcinogens causing DNA damage. When the stomach suffers from infection with Helicobacter Pylori (HP), HP lipopolysaccharides decrease mucin synthesis by the phosphatidylinositol 3-kinase/ERK pathway and via inhibition of galactosyltransferase (Slominay et al., 2003; Slominay et al., 2005). A large body of in vitro evidences indicate that treatment of gastric epithelial or adenocarcinoma cell lines with HP will cause the loss or reduced synthesis of mucin (Wang, et al., 2003; Durai Babu et al., 2006). In vivo studies have also suggested that HP infection is positively correlated with low expression of some mucins during the pathogenesis and development of gastric carcinomas (Byrd et al. 2000; Kim et al. 2003). Proinflammatory and inflammatory cytokines (IL-1, IL-6 and TNFα) trigger MUC-2, MUC-4 and MUC-5AC expression in gastrointestinal cancers (Enss et al., 2000; Mejías-Luque et al., 2008; Mejías-Luque et al., 2010). Therefore, altered mucin expression might be a key molecular event in gastric carcinogenesis. In previous work, we found that down-regulated MUC-6 expression was linked to gastric carcinogenesis, and subsequent progression of Japanese gastric cancer, while the converse was true for MUC-1 (Zheng et al., 2006; Li et al., 2008). Japan is within the high-risk area for 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 (Inoue et al., 2005). In the present study, we aimed to study the clinicopathological and prognostic significance of MUC2, MUC-4 and MUC-5AC expression in Japanese gastric carcinomas.

Materials and Methods

Patients

This retrospective study was carried out on curatively-resected gastric cancer specimens collected in Toyama University Hospital from 1993 to 2006. The patients with gastric carcinomas were 132 men and 299 women (38-88 years, mean=66.7 years). Archival materials were obtained from Department of Pathology. In 168 cases, tumor development was accompanied with lymph node metastasis. None of the patients underwent chemotherapy, radiotherapy or adjuvant treatment before surgery. 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 (Sobin et al., 2002). Histological architecture was defined in terms of Lauren's classification (Zheng et al., 2007; 2008). 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 two mm diameter tissue cores per donor block were punched out and transferred to a recipient block with a maximum 48 cores using a Tissue Microarrayer (AZUMAYA KIN-1, Japan). Four-μm-thick sections were consecutively cut from the microarrays and transferred to poly-lysine-coated glass slides. HE staining was performed for confirmation of tumor tissue.

Immunohistochemistry

Serial sections of TMA were deparaffinized with xylene, rehydrated with alcohol, and subjected to immunonhistochemical staining with intermittent microwave radiation as previously described (Kumada et al., 2004). Mouse anti-human MUC-2, MUC-4, and MUC-5AC antibodies (NovoCastra, UK) 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 and counterstaining with Mayer's hematoxylin was performed to aid orientation. Omission of the primary antibody was used as a negative contr ol.

Immunoreactivity for MUC-2 and MUC-5AC showed a cytoplasmic pattern, while MUC-4 was localized in the cytoplasm and membrane (Figure 1). One hundred cells were randomly selected and counted from five representative fields of each section blindly by twoindependent observers (Xiao and Zheng). The inconsistent data were confirmed by both persons until final agreements were reached. The expression positivity was graded and counted as follows: 0 =negative; 1 = 1-50%; 2 = 50-74%; 3 ≥75%. The staining intensity score was graded as follows: 1 = weak; 2 = intermediate; and 3 = strong. The scores for MUC-2, MUC-4 or MUC-5AC positivity and staining intensity were multiplied to obtain a final score, which determines their expression as (- = 0; + = 1-2; ++ = 3-4; +++ = 6-9).

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. SPSS 17.0 software was applied to analyze all data and $p<0.05$ was considered statistically significant.

Results

As indicated in Figure 1, MUC-2 was expressed in goblet cells of intestinal metaplasia. There was strong diffuse expression of MUC-2 in the cytoplasm of gastric carcinoma cells. MUC-2 was positively expressed in 67.4%(62/92) of gastric non-neoplastic mucosa (NNM) and 27.4%(131/431) of gastric cancer, respectively. Statistically, there was MUC-2 overexpression in gastric NNM than carcinoma (p<0.05, Figure 2A). As shown in Table 1, MUC-2 expression was negatively correlated with tumor size, depth of invasion, and TNM staging of gastric cancer (p<0.05). There was higher MUC-2 expression in intestinal- than diffuse-type carcinomas (p<0.05).

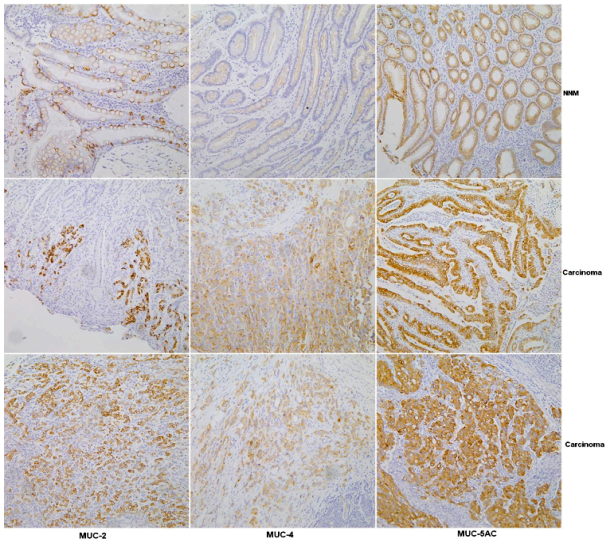

Figure 1. The in Situ Expression of MUC-2, MUC-4 and MUC-5AC Protein in Gastric Cancer. Note: The strong positivity of MUC-2 or MUC-5AC was localized in the cytoplasm, while MUC-4 in the membrane and cytoplasm of gastric intestinal metaplasia of non-neoplastic mucosa (NNM) and cancer

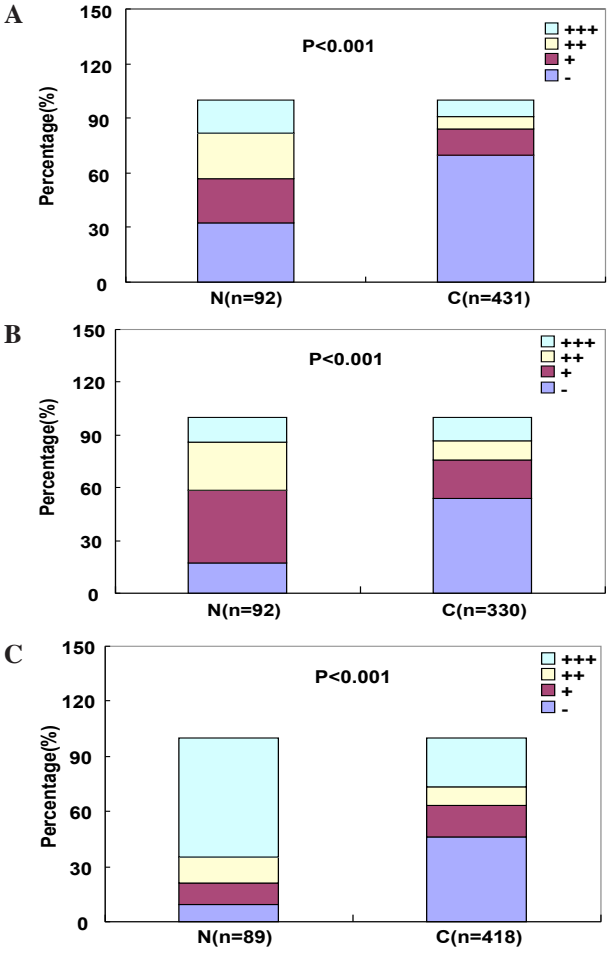

Figure 2. The Ratio Distribution of Different MUC-2, MUC-4 and MUC-5AC Expression Levels in Gastric Cancer. There was negative(-), weak(+), moderate(++) and strong(+++) expression of MUC-2, MUC-4 and MUC-5AC

Table 1. Relationship Between MUC-2 Expression and Clinicopathological Features of Gastric Carcinomas

Clinicopathological		MUC-2 expression					
features	n	-	+	++	+++	PR(%)	P value
Age(year)							0.058
<65	189	139	28	8	14	26.5	
≥65	242	161	35	22	24	33.5	
Sex							0.712
Male	132	93	16	8	15	29.5	
Female	299	207	47	22	23	30.8	
Tumor size(cm)							0.003
<4	223	145	41	18	19	35.0	
≥4	208	155	22	12	19	25.5	
Depth of invasion							0.002
T_{is-1}	217	141	44	19	13	35.0	
T_{2-4}	214	159	19	11	25	25.7	
Lymphatic invasion							0.230
-	271	188	46	19	18	30.6	
+	159	112	17	11	19	29.6	
Venous invasion							0.080
-	370	252	58	26	34	31.9	
+	61	48	5	4	4	21.3	
Lymph node metastasis							0.252
-	259	178	44	21	16	31.3	
+	168	120	18	8	22	28.6	
TNM staging							0.002
0-I	245	160	50	20	15	34.7	
II-IV	186	140	13	10	23	24.7	
Lauren's classification							<0.001
Intestinal-type	212	135	43	20	14	36.3	
Diffuse-type	205	156	18	9	22	23.9	
MUC-5 expression							0.011
-	189	141	27	7	14	25.4	
+	64	38	12	10	4	40.6	
++	42	28	6	3	5	33.3	
+++	106	70	16	7	13	34.0	

PR, positive rate; T_{is}, carcinoma in situ; T_1, lamina propria and submucosa; T_2, muscularis propria and subserosa; T_3, exposure to serosa ; T_4, invasion into serosa; TNM, tumor-node-metastasis

As indicated in Figure 1, MUC-4 was positively expressed in goblet cells of intestinal metaplasia, superficial epithelium and gastric carcinoma cells. There was strong diffuse expression of MUC-4 in the cytoplasm and membrane. MUC-4 was positively expressed in 82.3% (76/92) of gastric NNM and 46.1% (152/330) of gastric cancer, respectively (Figure 1B). Statistically, there was MUC-4 overexpression in gastric NNM than carcinoma (p<0.05, Figure 2B). MUC-4 expression was higher in the elder than the younger patients with gastric cancer (p<0.05, Table 2).

As indicated in Figure 1, MUC-5AC was expressed in goblet cells of intestinal metaplasia and gastric carcinoma cells. MUC-5AC was positively expressed in 91.0(81/89)

Table 2. Relationship Between MUC-4 Expression and Clinicopathological Features of Gastric Carcinomas

Clinicopathological		MUC-4 expression					
features	n	-	+	++	+++	PR(%)	*P* value
Age(year)							0.016
<65	129	79	19	14	17	38.8	
≥65	201	99	52	24	26	50.7	
Sex							0.075
Male	242	137	51	29	25	43.4	
Female	88	41	20	9	18	53.4	
Tumor size(cm)							0.361
<4	177	98	39	22	18	44.6	
≥4	153	80	32	16	25	47.7	
Depth of invasion							0.242
T_{is-1}	178	88	48	24	18	50.6	
T_{2-4}	152	90	23	14	25	40.8	
Lymphatic invasion							0.320
-	214	119	50	24	21	44.4	
+	115	58	21	14	22	49.6	
Venous invasion							0.941
-	291	157	63	33	38	46.0	
+	39	21	8	5	5	46.2	
Lymph node metastasis							0.369
-	210	115	52	23	20	45.2	
+	117	60	19	15	23	48.7	
TNM staging							0.802
0-I	205	110	51	24	20	46.3	
II-IV	125	68	20	14	23	45.6	
Lauren's classification							0.055
Intestinal-type	213	102	51	31	29	52.1	
Diffuse-type	109	70	19	7	13	35.8	
MUC-5AC							<0.001
-	135	91	22	10	12	32.6	
+	55	26	17	6	6	52.7	
++	34	13	7	10	4	61.8	
+++	86	35	21	11	19	59.3	

PR, positive rate; T_{is}, carcinoma in situ; T_1, lamina propria and submucosa; T_2, muscularis propria and subserosa; T_3, exposure to serosa ; T_4, invasion into serosa; TNM, tumor-node-metastasis

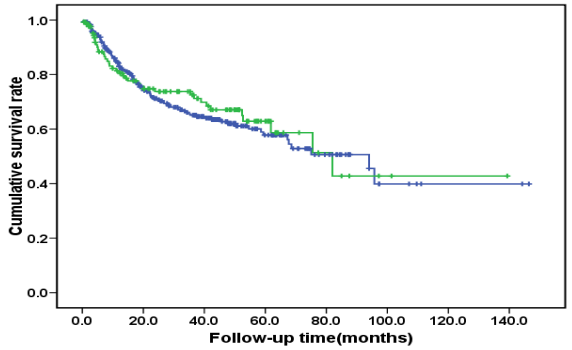

Figure 3. The Prognostic Significance of MUC-2 Expression in Patients with Gastric Cancer.

of gastric NNM and 53.6% (224/418) of gastric cancer, respectively. Statistically, there was MUC-5AC overexpression in gastric NNM than carcinoma ($p<0.05$, Figure 2C). As shown in Table 3, MUC-5AC expression was higher in the female than the male patients with gastric cancer ($p<0.05$). The older patients with carcinoma showed MUC-5AC overexpression, in comparison to the young ($p<0.05$). MUC-5AC expression was negatively associated with the depth of invasion, venous invasion,

Table 3. Relationship between MUC-5AC expression and clinicopathological features of gastric carcinomas

Clinicopathological		MUC-5 expression					
features	n	-	+	++	+++	PR(%)	P value
Age(year)							0.242
<65	175	84	29	15	47	52.0	
≥65	243	110	40	30	63	54.7	
Sex							0.024
Male	290	145	51	27	67	50.0	
Female	128	49	18	18	43	61.7	
Tumor size(cm)							0.358
<4	214	95	38	27	54	55.6	
≥4	204	99	31	18	56	51.5	
Depth of invasion							0.001
T_{is-1}	211	85	36	23	67	59.7	
T_{2-4}	207	109	33	22	43	47.3	
Lymphatic invasion							0.270
-	263	120	42	27	74	54.4	
+	154	74	27	17	36	51.9	
Venous invasion							0.017
-	361	160	58	43	100	55.7	
+	57	34	11	2	10	40.4	
Lymph node metastasis							0.012
-	252	109	44	25	74	56.7	
+	162	83	25	20	34	48.8	
TNM staging							0.002
0-I	238	98	40	25	75	58.8	
II-IV	180	96	29	20	35	46.7	
Lauren's classification							0.147
Intestinal-type	211	96	38	26	51	54.5	
Diffuse-type	199	97	27	18	57	51.3	

PR, positive rate; T_{is}, carcinoma in situ; T_1, lamina propria and submucosa; T_2, muscularis propria and subserosa; T_3, exposure to serosa ; T_4, invasion into serosa; TNM, tumor-node-metastasis

lymph node metastasis and TNM staging ($p<0.05$), but positively with MUC-4 and MUC-2 expression ($p<0.05$). Follow-up information was available on 431 of the gastric carcinoma patients for periods ranging from 0.2 months to 12.2 years (mean=70.8 months). Figure 3 shows a representative survival curve, stratified according to MUC-2 expression status. Univariate analyses using Kaplan-Meier method indicated no relationship between the three proteins' expression and cumulative survival rate of patients, even stratified by the depth of invasion ($p>0.05$).

Discussion

Mucins are high molecular weight O-linked glycoproteins whose primary functions are to hydrate, protect, and lubricate the epithelial luminal surfaces of the ducts within the human body (Zheng et al., 2006). Recent studies have uncovered the unique roles of mucins in the pathogenesis of cancer (Akyürek et al., 2002; Huang et al., 2002; Wang et al., 2003; Wang et al., 2003; Slomiany et al., 2003; Slomiany et al., 2005; Levi et al.2004; Cozzi et al.,2005; Li et al., 2006; Li et al., 2008; McGuckin et al., 2011). MUC-2 is particularly prominent in the gut where it is secreted from goblet cells in the epithelial lining into the lumen of the large intestine. Here, we found that MUC-2 expression was stronger in the intestinal metaplasia of

gastric NNM than gastric cancer in agreement with the data in ovarian carcinogenesis (Feng et al., 2002), indicating that down-regulated MUC-2 might play an important role in gastric carcinogenesis in agreement with another study (Bu et al., 2010). Additionally, MUC-2 expression was negatively correlated with tumor size, depth of invasion, and TNM staging of gastric cancer, in line with the previous reports (Rakha et al., 2005; İlhan et al., 2010), suggesting that reduced MUC-2 expression might be closely linked to the growth, invasion and progression of gastric cancer. The higher MUC-2 expression in intestinal- than diffuse-type carcinomas gives us a fact that it underlies the molecular mechanisms of the differentiation of both carcinomas, which is remarkably different from the findings of İlhan et al. (İlhan et al., 2010). Mesquita et al found that the promoter CpG methylation of MUC-2 was closely linked to its down-regulated expression in gastric cancer (Mesquita et al., 2003). A statistically significant association has been identified between rare exonic MUC2-MS6 alleles and the occurrence of gastric cancer, indicating that minisatellite instability might be associated with MUC2 function in cancer cells (Jeong et al., 2007).

MUC-4 is a high-molecular weight membrane glycoprotein and has been reported to play various roles in the progression of cancer, particularly due to its signaling and anti-adhesive properties which contribute to tumor development and metastasis (Carraway et al., 2009). In the present study, it was found that MUC-4 was distributed to the membrane of gastric superficial epithelium and cancer cells. Different from the results of Senapati et al., MUC-4 expression was higher in gastric cancer than its NNM, suggesting that down-regulated MUC-4 might be involved in gastric carcinogenesis (Senapati et al., 2008). Reportedly, epigenetic regulation of the human mucin gene MUC-4 in gastric and pancreatic cancer cell lines involves both DNA methylation and histone modifications mediated by DNA methyltransferases and histone deacetylases (Vincent et al., 2008). Shemirani et al. reported that MUC-4 were expressed 21-fold higher in stage I disease in mucoepidermoid carcinoma, compared to normal tissue by real-time PCR (Shemirani et al., 2011). MUC-4 overexpression is demonstrated in rat cholangiocarcinoma by tissue microarray (Yeh et al., 2009). However, the correlation of MUC-4 expression with aggressive behaviors was not observed in gastric cancer, consistent with other reports (Senapati et al., 2008; Kim et al., 2012), while Tamura et al. found that MUC-4 expression was related to lymphatic invasion and lymph node metastasis of gastric cancer(Tamura et al. 2012). Another group reported found that the increase in the expression and hypomethylation of MUC-4 gene with the progression of pancreatic ductal adenocarcinoma(Zhu et al., 2011).

MUC-5AC is a gel-forming mucin that is secreted from surface mucous cells. Glucocorticoid is required for the expression of Mucin-5AC mRNA and high doses of hydrocortisone suppress its expression (Takami et al., 2012). Mucin-5AC is also expressed in normal endocervical epithelium, small intestine, gastric cells (Lewis type 1) and gastric metaplasia, and it is a one of the major mucins in the ethmoid mucosa (Guillem et al., 2000; Jung et al., 2000). Here, we observed MUC-5AC expression in intestinal metaplasia and cancer cells and found its overexpression in NNM, compared with gastric cancer, suggesting an important role of MUC-5AC dowregulation in gastric carcinogenesis. Moreover, a negative link between MUC-5AC expression and aggressiveness of gastric cancer was found, including depth of invasion, venous invasion, lymph node metastasis and TNM staging, which was consistent with the findings about MUC-5AC in pancreatic invasive ductal carcinoma (Jinfeng et al., 2003) and from another group about gastric carcinoma (Wang et al., 2003). Yamazoe et al. (Yamazoe et al., 2010) found that knockdown of MUC-5AC reduced the ability of pancreatic cancer cells to adhesion and invasion, suggesting that MUC-5AC might contribute to the invasive motility of pancreatic cancer cells by enhancing the expression of integrins, MMP-3, VEGF and activating Erk pathway.

In the present study, we found no relationship between MUC-2, 4 or -5AC and the prognosis of the patients with gastric cancer. However, loss of expression of MUC-2 showed significant correlation with poor overall survival in colorectal carcinoma (Kang et al., 2011; Elzagheid et al., 2012). The survival analysis showed that MUC-4 expression was statistically significant risk factors affecting the outcome of the patients with intrahepatic cholangiocarcinoma-mass forming type as an independent risk factor (Shibahara et al., 2004). MUC 4-positive patients had a definite trend towards better survival of laryngeal squamous cancer (Paleri et al., 2004) and gallbladder carcinoma (Lee et al. 2012). In addition, patients with MUC-5AC- positive tumors also had poor clinicopathological parameters and showed shorter survival than those with MUC-5AC-negative gastric cancer (Kocer et al., 2004). MUC-5AC-positive patients showed significant better survival than those MUC5AC- negative patients with pancreatic invasive ductal carcinoma (Jinfeng et al., 2003). Therefore, the prognostic significance of MUC-2,-4, and -5AC in gastric cancer should need further investigation in the future.

Acknowledgements

This study was supported by Shenyang Science and Technology Grant (F11-264-1-10; F12-277-1-01); Liaoning Science and Technology Grant (2011225019); Natural Scientific Foundation of China (81101885; 81172371) and Liaoning Science and Technology Grant (2011225019).

References

Akyürek N, Akyol G, Dursun A, Yamaç D, Günel N (2002). Expression of MUC1 and MUC2 mucins in gastric carcinomas: their relationship with clinicopathologic parameters and prognosis. *Pathol Res Pract*, **198**, 665-74.

Bu XD, Li N, Tian XQ, et al (2010). Altered expression of MUC2 and MUC5AC in progression of colorectal carcinoma. *World J Gastroenterol*, **16**, 4089-94.

Byrd JC, Yunker CK, Xu QS, Sternberg LR, Bresalier RS (2000).

Inhibition of gastric mucin synthesis by Helicobacter pylori. *Gastroenterology*, **118**, 1072-9.
Carraway KL, Theodoropoulos G, Kozloski GA, Carothers Carraway CA (2009). Muc4/MUC4 functions and regulation in cancer. *Future Oncol*, **5**, 1631-40.
Cozzi PJ, Wang J, Delprado W, et al (2005). MUC1, MUC2, MUC4, MUC5AC and MUC6 expression in the progression of prostate cancer. *Clin Exp Metastasis*, **22**, 565-73.
Durai Babu S, Jayanthi V, Niranjali D, Reis CA, Devaraj H (2006). Expression profile of mucins (MUC2, MUC5AC and MUC6) in Helicobacter pylori infected pre-neoplastic and neoplastic human gastric epithelium. *Mol Cancer*, **5**, 10.
Elzagheid A, Emaetig F, Buhmeida A, et al (2012). Loss of MUC2 expression predicts disease recurrence and poor outcome in colorectal carcinoma. *Tumour Biol in press*.
Enss ML, Cornberg M, Wagner S, et al (2000). Proinflammatory cytokines trigger MUC gene expression and mucin release in the intestinal cancer cell line LS180. *Inflamm Res*, **49**, 162-9.
Feng H, Ghazizadeh M, Konishi H, Araki T (2002). Expression of MUC1 and MUC2 mucin gene products in human ovarian carcinomas. *Jpn J Clin Oncol*, **32**, 525-9.
Guillem P, Billeret V, Buisine MP, et al (2000). Mucin gene expression and cell differentiation in human normal, premalignant and malignant esophagus. *Int J Cancer*, **88**, 856-61.
Hollingsworth MA, Swanson BJ (2004). Mucins in cancer: protection and control of the cell surface. *Nat Rev Cancer*, **4**, 45-60.
Huang WB, Shi LH, Zhu XQ, et al (2002). Expression of mucin MUC1 and MUC2 in colorectal carcinoma and their clinical significance. *Ai Zheng*, **21**, 1231- 4.
İlhan Ö, Han Ü, Önal B, Çelık SY (2010). Prognostic significance of MUC1, MUC2 and MUC5AC expressions in gastric carcinoma. *Turk J Gastroenterol*, **21**, 345-52.
Inoue M, Tsugane S (2005). Epidemiology of gastric cancer in Japan. *Postgrad Med J*, **81**, 419-24.
Jeong YH, Kim MC, Ahn EK, et al (2007). Rare exonic minisatellite alleles in MUC2 influence susceptibility to gastric carcinoma. *PLoS One*, **2**, e1163.
Jinfeng M, Kimura W, Hirai I, et al (2003). Expression of MUC5AC and MUC6 in invasive ductal carcinoma of the pancreas and relationship with prognosis. *Int J Gastrointest Cancer*, **34**, 9-18.
Jung HH, Lee JH, Kim YT, Lee SD, Park JH (2000). Expression of mucin genes in chronic ethmoiditis. *Am J Rhinol*, **14**, 163-70.
Kang H, Min BS, Lee KY, et al (2011). Loss of E-cadherin and MUC2 expressions correlated with poor survival in patients with stages II and III colorectal carcinoma. *Ann Surg Oncol*, **18**, 711-9.
Lee HK, Cho MS, Kim TH (2012). Prognostic significance of muc4 expression in gallbladder carcinoma. *World J Surg Oncol in press*.
Kelley JR, Duggan JM (2003). Gastric cancer epidemiology and risk factors. *J Clin Epidemiol*, **56**, 1-9.
Kim H, Seo JH, Kim KH (2003). The effect of p38 mitogen-activated protein kinase on mucin gene expression and apoptosis in Helicobacter pylori-infected gastric epithelial cells. *Ann N Y Acad Sci*, **1010**, 90-4.
Kim SM, Oh SJ, Hur B (2012). Expression of MUC1 and MUC4 in gallbladder adenocarcinoma. *Korean J Pathol*, **46**, 429-35.
Kocer B, Soran A, Kiyak G, et al (2004). Prognostic significance of mucin expression in gastric carcinoma. *Dig Dis Sci*, **49**, 954-64.
Kumada T, Tsuneyama K, Hatta H, Ishizawa S, Takano Y (2004). 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**, 1141-9.
Levi E, Klimstra DS, Andea A, Basturk O, Adsay NV (2004). MUC1 and MUC2 in pancreatic neoplasia. *J Clin Pathol*, **57**, 456-62.
Li XH, Zheng HC, Wang ZG, et al (2008). The clinicopathological and prognostic significance of MUC-1 expression in Japanese gastric carcinomas: an immunohistochemical study of tissue microarrays. *Anticancer Res*, **28**, 1061-7.
McGuckin MA, Lindén SK, Sutton P, Florin TH (2011). Mucin dynamics and enteric pathogens. *Nat Rev Microbiol*, **9**, 265-78.
Mejías-Luque R, Lindén SK, Garrido M, et al (2010). Inflammation modulates the expression of the intestinal mucins MUC2 and MUC4 in gastric tumors.*Oncogene*, **29**, 1753-62.
Mejías-Luque R, Peiró S, Vincent A, Van Seuningen I, de Bolós C (2008). IL-6 induces MUC4 expression through gp130/STAT3 pathway in gastric cancer cell lines. *Biochim Biophys Acta*, **1783**, 728-36.
Mesquita P, Peixoto AJ, Seruca R, et al (2003). Role of site-specific promoter hypomethylation in aberrant MUC2 mucin expression in mucinous gastric carcinomas. *Cancer Lett*, **189**, 129-36.
Paleri V, Pearson JP, Bulmer D, et al (2004). Expression of mucin gene products in laryngeal squamous cancer. *Otolaryngol Head Neck Surg*, **131**, 84-8.
Rakha EA, Boyce RW, Abd El-Rehim D, et al (2005). Expression of mucins (MUC1, MUC2, MUC3, MUC4, MUC5AC and MUC6) and their prognostic significance in human breast cancer. *Mod Pathol*, **18**, 1295-304.
Rivera F, Vega-Villegas ME, López-Brea MF (2007). Chemotherapy of advanced gastric cancer. *Cancer Treat Rev*, **33**, 315-24.
Senapati S, Chaturvedi P, Sharma P, et al (2008).Deregulation of MUC4 in gastric adenocarcinoma: potential pathobiological implication in poorly differentiated non-signet ring cell type gastric cancer. *Br J Cancer*, **99**, 949-56.
Shemirani N, Osipov V, Kolker A, Khampang P, Kerschner JE (2011). Expression of mucin (MUC) genes in mucoepidermoid carcinoma. *Laryngoscope*, **121**, 167-70.
Shibahara H, Tamada S, Higashi M, et al (2004). MUC4 is a novel prognostic factor of intrahepatic cholangiocarcinoma-mass forming type. *Hepatology*, **39**, 220-9.
Slomiany BL, Slomiany A (2005).Up-regulation in endothelin-1 by Helicobacter pylori lipopolysaccharide interferes with gastric mucin synthesis via epidermal growth factor receptor transactivation. *Scand J Gastroenterol*, **40**, 921-8.
Slomiany B, Slomiany A (2003). 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.
Sobin LH, Wittekind CH (2002). TNM Classification of malignant tumors, 6th edn. *New Jersey: John Wiley & Sons, Hoboken*.
Takami S, Mizuno T, Oyanagi T, et al (2012). Glucocorticoids inhibit MUC5AC production induced by transforming growth factor-α in human respiratory cells. *Allergol Int*, **61**, 451-9.
Tamura Y, Higashi M, Kitamoto S, et al (2012). MUC4 and MUC1 expression in adenocarcinoma of the stomach correlates with vessel invasion and lymph node metastasis: an immunohistochemical study of early gastric cancer. *PLoS One*, **7**, e49251.
Vincent A, Ducourouble MP, Van Seuningen I (2008). Epigenetic regulation of the human mucin gene MUC4 in epithelial

cancer cell lines involves both DNA methylation and histone modifications mediated by DNA methyltransferases and histone deacetylases. *FASEB J*, **22**, 3035-45.

Wang JY, Chang CT, Hsieh JS, et al(2003) Role of MUC1 and MUC5AC expressions as prognostic indicators in gastric carcinomas. *J Surg Oncol*, **8**, 253-60.

Wang RQ, Fang DC (2003). Alterations of MUC1 and MUC3 expression in gastric carcinoma: relevance to patient clinicopathological features. *J Clin Pathol*, **56**, 378-84.

Yamazoe S, Tanaka H, Sawada T, et al (2010). RNA interference suppression of mucin 5AC (MUC5AC) reduces the adhesive and invasive capacity of human pancreatic cancer cells. *J Exp Clin Cancer Res*, **29**, 53.

Yeh CN, Pang ST, Wu RC, et al (2009). Prognostic value of MUC4 for mass-forming intrahepatic cholangiocarcinoma after hepatectomy. *Oncol Rep*, **21**, 49-56.

Zheng HC, Li XH, Hara T, et al (2008). Mixed-type gastric carcinomas exhibit more aggressive features and indicate the histogenesis of carcinomas.*Virchows Arch*, **452**, 525-34.

Zheng H, Takahashi H, Murai Y, et al (2007). Pathobiological characteristics of intestinal and diffuse-type gastric carcinoma in Japan: an immunostaining study on the tissue microarray. *J Clin Pathol*, **60**, 273-7.

Zheng H, Takahashi H, Nakajima T, et al (2006).MUC6 down-regulation correlates with gastric carcinoma progression and a poor prognosis: an immunohistochemical study with tissue microarrays. *J Cancer Res Clin Oncol*, **132**, 817-23.

Zhu Y, Zhang JJ, Zhu R, et al (2011). The increase in the expression and hypomethylation of MUC4 gene with the progression of pancreatic ductal adenocarcinoma. *Med Oncol*, **28**, S175-84.