ORIGINAL ARTICLE

Alterations of MUC1 and MUC3 expression in gastric carcinoma: relevance to patient clinicopathological features

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Aims: Several studies have reported conflicting and inconclusive results concerning the clinical relevance of mucin expression in gastric carcinoma. This study investigated the correlations between aberrant expression of mucins in gastric carcinoma and patient clinicopathological features.
Methods: The expression of MUC1, MUC2, MUC3, MUC5AC, and MUC6 was investigated immunohistochemically in gastric carcinoma (n = 46) in relation to patient clinicopathological features.
Results: All normal gastric mucosa samples expressed MUC1, MUC5AC, and MUC6. MUC1, MUC2, MUC3, MUC5AC, and MUC6 were expressed in 29, 31, 30, 18, and 21 of the 46 cases of gastric carcinoma, respectively. The number of cases expressing MUC1 was significantly higher ($p < 0.01$) in patients with a small tumour size (≥ 5 cm) and in patients in clinical stages I–II, compared with clinical stages III–IV ($p < 0.05$). Expression was significantly lower ($p < 0.05$) in patients exhibiting metastasis. The number of cases expressing MUC3 was significantly higher in patients in clinical stages III–IV ($p < 0.05$), and in those with serosal invasion ($p < 0.05$) or metastasis ($p<0.01$). No significant relations were found between MUC2, MUC5AC, MUC6, and clinical stage, metastasis, or tumour size.
Conclusions: Membrane bound mucins MUC1 and MUC3 appear to be associated with the development of gastric carcinoma. Patients who maintained high immunoreactivity for anti-MUC1 antibody had a better prognosis, whereas those with an increase in anti-MUC3 immunoreactivity had a poorer prognosis, as judged by tumour size, serosal invasion, and metastasis. However, no correlation was found between MUC2, MUC5AC, or MUC6 and clinical prognosis.

Epithelial mucins can be classified into two distinct families: secretory (gel forming) and membrane bound. Members of each family possess common structural characteristics and at least some common functions. In general, mucins have the unique function of protecting and lubricating epithelial surfaces, but in recent years they have also been implicated in additional diverse roles, such as growth, fetal development, epithelial renewal and differentiation, epithelial integrity, carcinogenesis, and metastasis.

Aberrant (upregulated) expression of the MUC1 mucin has been seen in breast carcinomas and other neoplasms, such as colon and pancreatic cancers.[1 2] Most studies of MUC1 expression in breast carcinomas have shown that increased membrane MUC1 mucin expression on the apical cell surface is associated with a better prognosis (reviewed in Rahn and colleagues[2]), whereas circumferential staining in tumour cell cytoplasm is associated with a worse prognosis. These findings may be linked to reports that MUC1 plays an important role in the impairment of cell–cell adhesion, the immune response, and/or altered intracellular signalling.[3 4] Several tumour vaccines directed against MUC1 core protein, oligosaccharides, or MUC1 cDNAs have been shown to stimulate immune reactions in basic clinical trials to confer antitumour activity.[5–8]

There are at least five intestinal membrane mucins; namely, MUC3, MUC4, MUC12, MUC13, and the recently described mucin MUC17, which share a C-terminal structural domain that consists of two or three epithelial growth factor (EGF)-like regions, a transmembrane segment, and a short cytoplasmic tail. Rat Muc4, a homologue of human MUC4, has been extensively studied in mammary gland tumours and has been shown to play an important role in epithelial growth, cell differentiation, cell–cell adhesion, metastasis, and tumour apoptosis. The first EGF-like domain of rMuc4 is reported to be an intramembrane modulator of the c-erbB-2 receptor tyrosine kinase.[9] Structural similarities among membrane mucins suggest that they may subserve common functional properties. However, the expression of membrane associated mucins in gastric carcinomas has not been explored extensively. Recent reports by Lee *et al* have shown that changes of MUC1 mucin in gastric carcinomas may be related to patient prognosis,[10] but the importance of MUC1 in the progression of gastric carcinoma remains unresolved. There are a few published reports of intestinal MUC3 expression in colon and lung cancers,[11–13] but the expression and importance of MUC3 have also not been investigated in gastric cancers.

> "The expression of membrane associated mucins in gastric carcinomas has not been explored extensively"

Secretory mucins MUC2, MUC5AC, and MUC6 have been reported to be aberrantly expressed during the development of gastric carcinoma.[14] In the new grading system that combines intracellular mucin content and tubular differentiation (Goseki's classification[15]), an attempt was made to relate prognosis to the mucin content of the carcinoma. It was found that the frequency and extent of haematogenous metastasis to the liver—for example—was high in group I patients who had well defined tubular differentiation and low mucous content in the cytoplasm, whereas in group IV patients who had poor tubular differentiation and higher concentrations of mucous in the cytoplasm, lymph node metastasis, direct invasion into

Abbreviations: EGF, epithelial growth factor; PBS, phosphate buffered saline

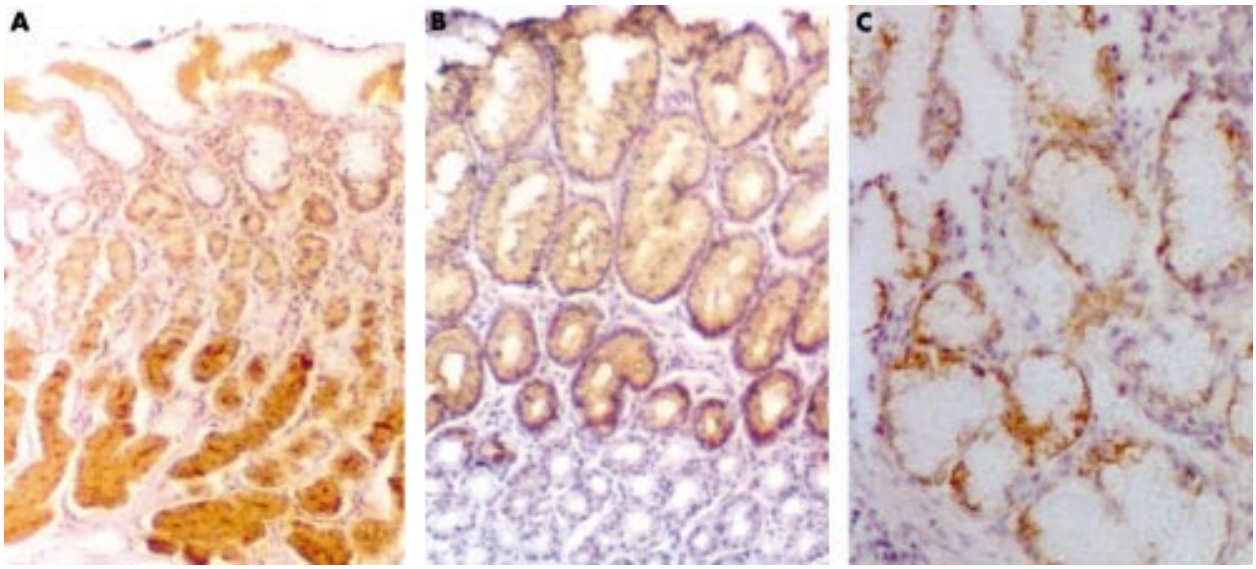

Figure 1 Immunohistochemical staining of normal gastric mucosa. (A) MUC1 detected in the superficial epithelium and antral gland (original magnification, ×100); (B) MUC5AC detected in foveolar epithelial cells (original magnification, ×100); (C) MUC6 detected in mucous cells of the neck zone and antral glands (original magnification, ×100).

surrounding organs, and/or peritoneal dissemination were higher.[15] However, a question that remains to be answered is whether membrane associated mucins or secretory mucins can provide the best indicator of prognosis.

The purpose of our present study was to determine whether the expression of representative membrane associated and/or secretory mucins in gastric carcinoma was associated with key clinicopathological features of the tumours. These studies are expected to assist in distinguishing potential roles played by secretory mucins versus membrane associated mucins in the development of gastric cancer, and in predicting clinical prognosis.

METHODS

Patients and clinicopathological information

Forty six patients who had undergone surgical resections of gastric carcinoma at the Southwest Hospital, PR China, were selected for our study. Age, sex, tumour location, tumour size, histological proliferation, clinical stage, and pathological data, such as depth of invasion and presence of metastasis, were obtained from hospital records. Clinical stages were based on the international standard TNM method (according to the World Health Organisation). The study cohort consisted of 34 men and 12 women (mean age, 54.6 years; range, 30–70). Formalin fixed, paraffin wax embedded sections of histologically corroborated gastric carcinoma (n = 46), histologically normal mucosa (n = 10), and pericancerous mucosa (at least 5 cm adjacent to the carcinoma; n = 39) were assessed. Of the 39 specimens obtained from pericancerous mucosa, 29 showed evidence of intestinal metaplasia. The sections were independently examined and diagnosed by two pathologists.

The Lauren type of gastric carcinoma was determined by the following criteria: the intestinal type of gastric carcinoma is histologically characterised by the presence of cohesive cells forming glandular and papillary structures and acidic mucous (as judged by Alcian blue (pH 2.5)/periodic acid Schiff staining of mucous); the diffuse type of gastric carcinoma is characterised histologically by non-cohesive cells, the common presence of signet ring cells, and neutral mucous (as judged by high iron diamine/Alcian blue (pH 2.5) staining); the mixed type of gastric carcinoma has both of the above characteristics, and both acidic and neutral mucous.

Immunohistochemistry

Tissue sections were dewaxed, rehydrated, incubated with 3% hydrogen peroxide in methanol for 30 minutes to abolish endogenous peroxidase activities, and then washed with 1× phosphate buffered saline (PBS), pH 7.4. After incubation with non-immune horse serum for 30 minutes at room temperature, the sections were washed and incubated with mucin specific antibodies overnight at 4°C. Biotin labelled sheep antimouse antibody or biotin labelled goat antirabbit antibody (1/100 dilution; Vector Laboratories, Burlingame, California, USA) were used as the secondary antibody. After incubation for 30 minutes, sections were treated with streptavidin–biotin peroxidase (Vector Laboratories) for 30 minutes and the reaction product visualised with 3,3′-diaminobezidine tetrahydrochloride (Zymed Laboratories, San Francisco, California, USA) and H_2O_2. Sections were lightly counterstained with haematoxylin. Negative controls included substitution of the primary antibodies with PBS buffer or unrelated antibodies.

Mucin specific antibodies included monoclonal antibody BC1 (1/100 dilution), which recognises human MUC1 core peptide and carbohydrate[16 17]; monoclonal antibody CCP58 (1/150 dilution), which recognises synthetic tandem repeat consensus peptide and is specific for human MUC2 apomucin[18]; monoclonal antibody SM-3 (1/100 dilution), which is specific for the synthetic tandem repeat consensus peptide KTTSNSTPSFTSSITTTETTSHS of human MUC3[19]; monoclonal antibody 45M1 (1/100 dilution; Neomarker Corp, Freemont, California, USA), which recognises MUC5AC non-glycosylated apomucin; and rabbit anti-MUC6 polyclonal antibody (1/200 dilution), which was raised against the synthetic polypeptide SFQTTTTYPTPSHPQTTLP, within the tandem repeat of MUC6.[20]

To obtain a more precise relation between mucin and prognostic indicators, a semiquantitative analysis was performed of mucin expressing cells in the total tumour bearing population. We reviewed five to 10 fields of each cancerous tissue (n = 29) at high magnification (×400) and counted the number of cells in each field expressing different mucins. The analysis was graded as +++ if 50–100% of cancer cells stained positive for mucins, ++ if 10–50% cancer cells stained positive, and + if less than 10% of cancer cells were positive for mucin staining. Patients were then categorised into group 1 (+), group 2 (++), or group 3 (+++).

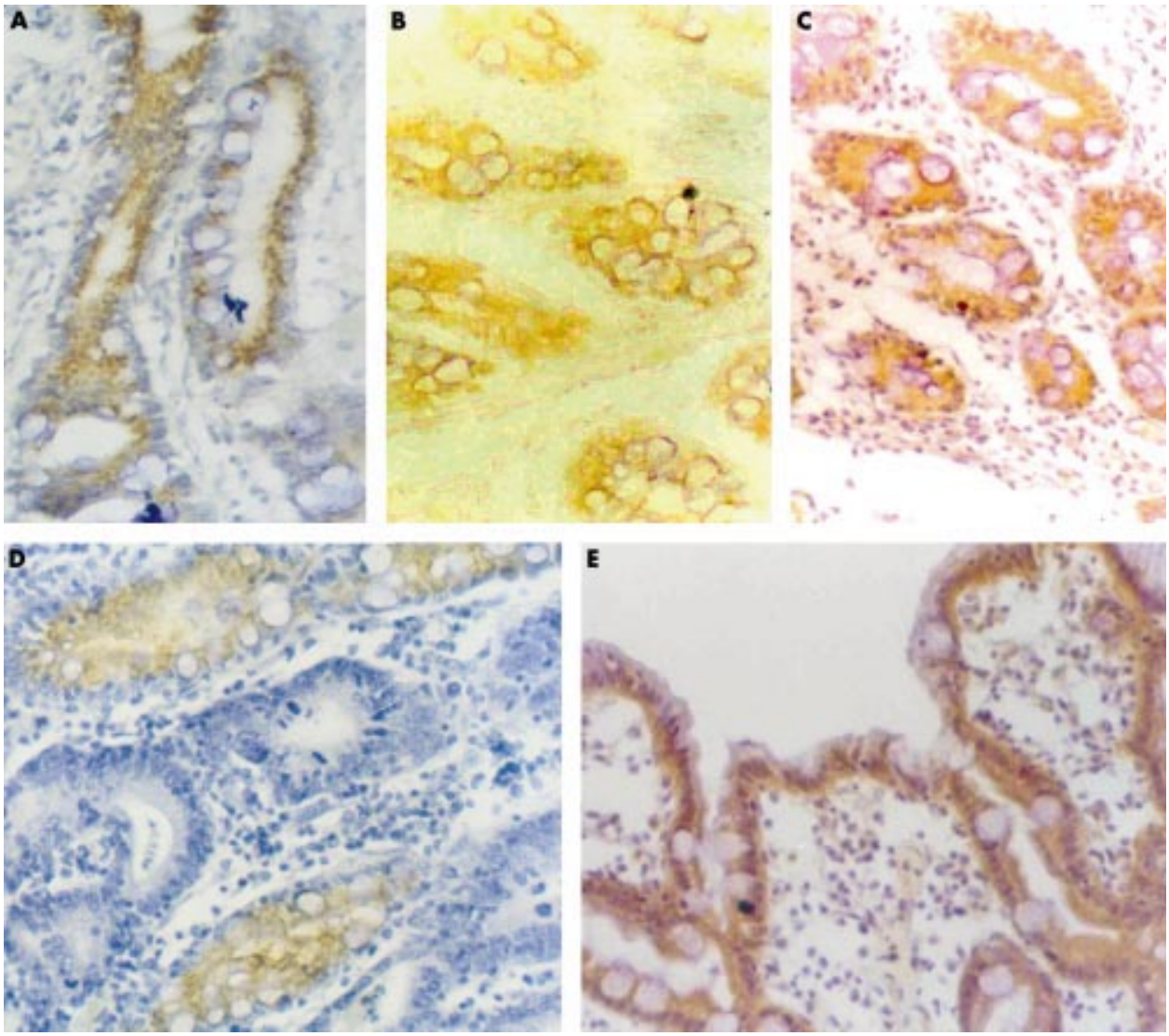

Figure 2 Distribution of MUC1, MUC2, MUC3, MUC5AC, and MUC6 mucin by immunohistochemistry in sections of intestinal metaplasia. (A) MUC1, (D) MUC5AC, and (E) MUC6 were located in the perinuclear area of positive staining cells (original magnification, ×400). (B) MUC2 and (C) MUC3 were located in the cytoplasm of positive staining cells (original magnification, ×400).

Statistical analysis

A χ^2 test was used to analyse the data. Differences were considered significant if p values were < 0.05, and highly significant if p was < 0.01.

RESULTS

Mucin products in normal gastric mucosa, intestinal metaplasia, and gastric carcinoma

The expression of the mucin genes was assessed by immunohistochemistry. In all 10 sections of normal gastric mucosa studied, abundant amounts of MUC1, MUC5AC, and MUC6 were detected. However, each mucin differed in its expression pattern (fig 1). Immunoreactivity for MUC1 (fig 1A) and MUC5AC (fig 1B) was detected in the superficial foveolar epithelium, whereas MUC6 (fig 1C) was expressed in the mucous cells of the neck zone and antral glands. MUC2 and MUC3 were not detected in the normal mucosa.

Figure 2 shows typical immunohistochemical results for the mucins in gastric mucosa undergoing intestinal metaplasia. Although positive staining was seen for all mucins, the staining pattern for each mucin was distinct. MUC2 staining was very weak and was found only in goblet cells, whereas the other mucins were expressed in both goblet and columnar cells. Furthermore, staining for MUC1, MUC5AC, and MUC6 mucins was restricted to the perinuclear regions of the cells, whereas staining for MUC2 and MUC3 was seen in the cytoplasm. None of the mucin antibodies gave a positive signal in the area of mucous granules. Of all the cases of intestinal metaplasia examined by immunohistochemistry (a total of 29), 23 were positive for MUC1, 25 for MUC2, and 26 for MUC3. In contrast, MUC5AC and MUC6 expression was seen in only eight and four samples, respectively. Thus, a shift in mucin expression was characteristic of intestinal metaplasia, including an increase in the expression of MUC2 and MUC3 and a decrease in MUC1, MUC5AC, and MUC6.

Figure 3 shows typical immunohistochemical results for the mucins in gastric carcinoma (46 cases). In the case of mucin MUC1 (fig 3A), MUC5AC (fig 3D), and MUC6 (fig 3E), staining appeared in clusters within the sections, whereas immunostaining for MUC2 (fig 3B) and MUC3 (fig 3C) was more diffuse. Samples from a total of 29 of the 46 patients stained positive for MUC1, 31 for MUC2, and 30 for MUC3. Eighteen and 21 cases were positive for MUC5AC and MUC6, respectively. The shifts in mucin expression were not as dramatic as those seen for intestinal metaplasia. This may only be a function of the higher number evaluated in the group with gastric carcinoma. However, the trend was similar, with a

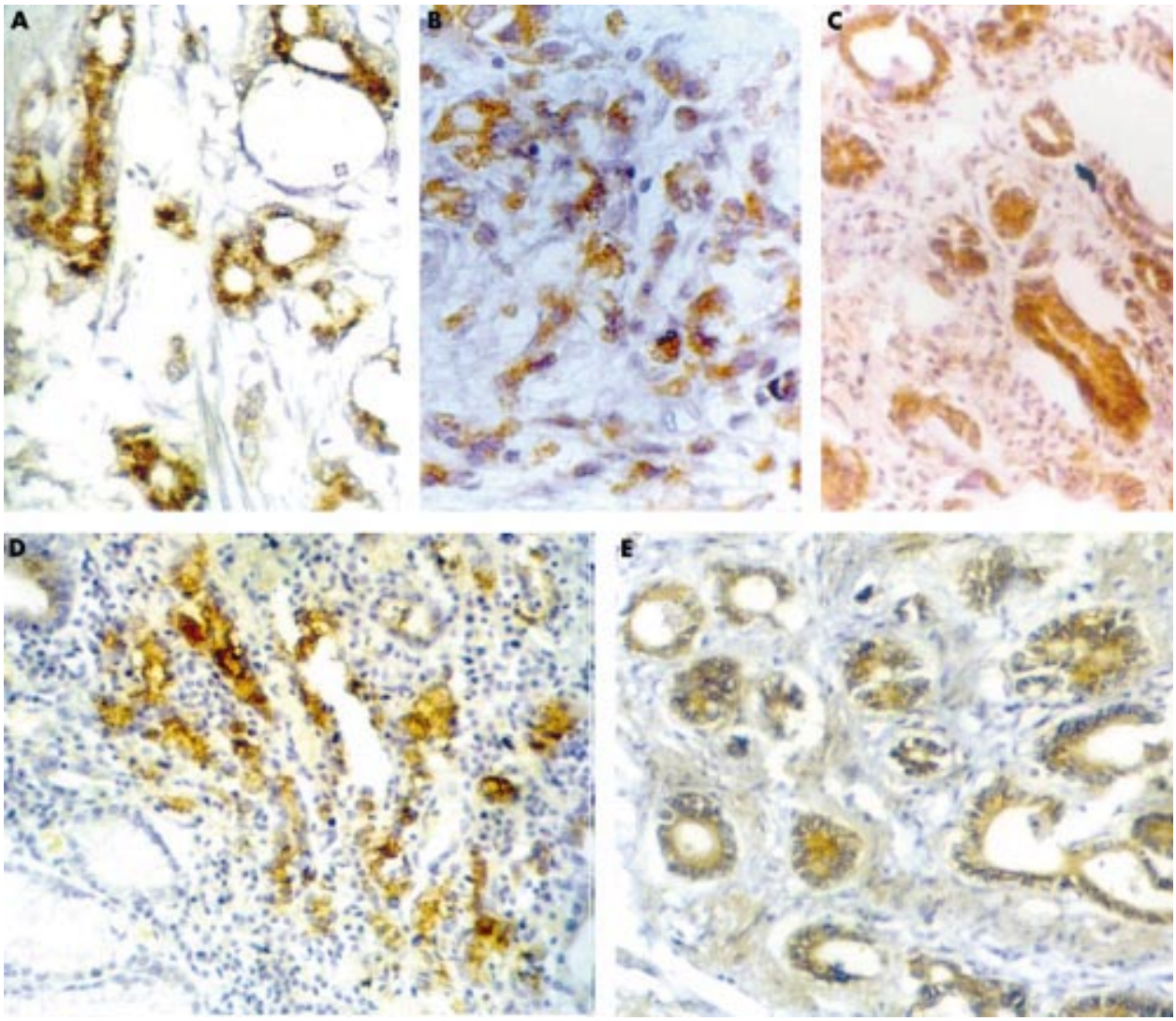

Figure 3 Immunohistochemical localisation of MUC1, MUC2, MUC3, MUC5AC, and MUC6 in gastric carcinoma. (A) MUC1, (D) MUC5AC, and (E) MUC6 exhibited a clustered staining pattern (original magnification, ×200), whereas (B) MUC2 (original magnification, ×400) and (C) MUC3 (original magnification,, ×200) showed a diffuse staining pattern.

decrease in the normal gastric mucins MUC1, MUC5AC, and MUC6, and an increase in "aberrant" expression of MUC2 and MUC3.

Expression of MUC1 and MUC3 correlates with tumour size, invasion, metastasis, and clinical stage of the disease

In patients with gastric tumours, there was no significant difference in immunoreactivity for the MUC1 mucin with respect to patient sex, tumour location, grade of differentiation, serosal invasion, or Lauren's type ($p > 0.05$) (table 1). However, the number of cases expressing MUC1 was significantly higher ($p<0.01$) in patients with a small ($\leqslant 5$ cm) versus a large (> 5 cm) tumour size. The number was also significantly higher ($p < 0.05$) in the group of patients within clinical stages I–II than in those in clinical stages III–IV. The number of cases expressing MUC1 was significantly lower ($p < 0.05$) in the group of patients exhibiting metastasis.

To obtain a more precise relation between mucin and prognostic indicators, a semiquantitative analysis was performed of mucin expressing cells in the total tumour bearing population. As shown in fig 4, the number of patients with metastasis was found to be significantly lower in group 3 (high MUC1 staining) than in groups 1 and 2 ($p < 0.01$). When the three groups were compared with respect to the clinical stage of the disease, group 3 also exhibited a significantly higher number of patients that were in clinical stage I–II than did groups 1 and 2 ($p < 0.01$). Thus, MUC1 mucin immunoreactivity in gastric carcinoma was highest in patients judged to have the best prognosis (small tumour size, lower clinical staging, and absence of metastasis).

Despite the prominence of aberrant MUC3 in gastric tumours, there was no significant difference in MUC3 immunoreactivity with respect to patient sex, tumour location, grade of differentiation, serosal invasion, or Lauren's type (table 1). However, the number of cases expressing MUC3 was significantly higher ($p < 0.01$) in the group with metastasis than in those without metastasis. Similarly, the number of cases expressing MUC3 was also found to be significantly higher ($p < 0.05$) in the group in clinical stage III–IV or with serosal invasion than in those in clinical stages I–II or without serosal invasion. Thus, tumours expressing MUC3 appear to correlate with unfavourable prognostic indicators.

We carried out semiquantitative analysis of MUC3 expressing cells in the total tumour bearing population (as for MUC1), but no relation was found between the different groups of MUC3 expression and prognostic indicators (results not shown).

Table 1 Association between the expression of MUC1 and MUC3 in cancer tissues and the clinicopathological features of the patient

Variables	n	MUC1 (+)	n	MUC3 (+)
Sex				
Male	34	23 (67.6%)	34	23 (67.6%)
Female	12	6 (50.0%)	11	7 (63.6%)
p Value		NS		NS
Tumour location				
Fundus	7	6 (85.7%)	7	3 (42.9%)
Body	13	6 (46.2%)	13	9 (69.2%)
Antrum	26	17 (65.4%)	25	18 (72.0%)
p Value		NS		NS
Size				
≤5 cm	31	24 (77.4%)	31	21 (67.7%)
>5 cm	15	5 (33.3%)	14	9 (64.3%)
p Value		<0.01		NS
Differentiation				
Well/moderate	26	17 (65.4%)	26	20 (76.9%)
Poor	17	10 (58.8%)	16	8 (50.0%)
Mucoid	3	2 (66.7%)	3	2 (66.7%)
p Value		NS		NS
Metastasis				
Present	23	11 (47.8%)	23	20 (87.0%)
Absent	23	18 (78.3%)	22	10 (45.5%)
p Value		<0.05		<0.01
Serosal invasion				
Present	26	16 (61.5%)	25	20 (80.0%)
Absent	20	13 (65.0%)	20	10 (50.0%)
p Value		NS		<0.05
Clinical stage				
I–II	26	21 (80.8%)	25	12 (48.0%)
III–IV	20	8 (40.0%)	20	18 (90.0%)
p Value		<0.01		<0.05
Types of carcinoma				
Intestinal	21	13 (61.9%)	20	12 (60.0%)
Diffuse	17	11 (64.7%)	17	11 (64.7%)
Mixed	8	5 (62.5%)	8	7 (87.5%)
p Value		NS		NS

NS, not significant.

Expression of MUC2, MUC5AC, and MUC6 in gastric carcinoma does not correlate with clinical stage, metastasis, or local invasion of disease

No significant difference in the expression of mucins MUC2, MUC5AC, or MUC6 was seen in the cancerous tissues among the different groups of patients studied, with respect to patient sex, tumour location, grade of differentiation, serosal invasion, or metastasis (table 2). Furthermore, no association was found between the different groups of MUC2, MUC5AC, and MUC6 expression and prognostic indicators of patients based on semiquantitative analysis (results not shown).

As judged by the Lauren classification system (see Methods), patients were grouped into three types; namely, intestinal, diffuse, and mixed type (table 2). There was no significant difference in the expression of MUC2 and MUC6 among the three Lauren types. However, the expression of MUC5AC was found to be significantly higher ($p < 0.05$) in the mixed type than in the other two types.

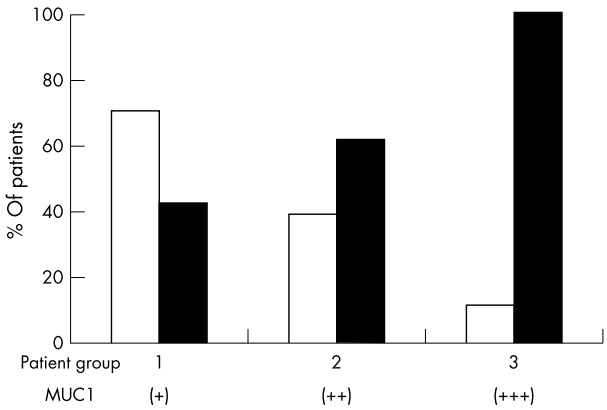

Figure 4 Relation between the intensity of MUC1 staining in cancer tissues, metastasis, and clinical stages of gastric tumour. The Y axis shows the per cent of patients in each group. Patients were divided into groups 1, 2, 3 (+, ++, +++, respectively) on the basis of MUC1 staining in tumour tissues. Groups 1, 2, and 3 contained seven, 13, and nine patients, respectively. White bars, percentage of patients with metastases; black bars, percentage of patients in clinical stage I–II.

DISCUSSION

We have described the profile of currently known gastric mucin genes found in normal gastric mucosa, intestinal metaplasia, and gastric carcinoma. An attempt was made to elucidate possible correlations between mucin translation products and patient clinicopathological features in the development of gastric carcinoma. This information is needed to provide insight into the possible role of mucins, especially the membrane tethered mucins MUC1 and MUC3, in prognosis. MUC1 has been studied relatively extensively in non-gastric but not in gastric tumours, but very little is known about the functions of MUC3 during carcinogenesis.[21]

Using the BC1 antibody, raised against human milk fat globule membrane and thought to recognise both MUC1 core peptide and normal carbohydrate epitopes of MUC1,[16 17] we found that higher MUC1 immunoreactivity was associated with a small tumour size (≤ 5 cm), absence of metastasis, and early clinical staging (I–II). Because these features are important prognostically, we conclude that high MUC1 mucin expression in gastric carcinoma may be a good prognostic indicator.

Table 2 Association between the expression of MUC2, MUC5AC, and MUC6 in cancer tissues and the clinicopathological features of the patients

Variables	n	MUC2 (+)	MUC5AC (+)	MUC6 (+)
Sex				
Male	34	24 (70.6%)	15 (44.1%)	16 (47.1%)
Female	12	7 (58.3%)	3 (27.3%)	5 (41.7%)
p Value		NS	NS	NS
Tumour location				
Fundus	7	6 (85.7%)	4 (66.7%)	3 (42.9%)
Body	13	9 (69.2%)	3 (23.1%)	8 (61.5%)
Antrum	26	16 (61.5%)	11 (42.3%)	10 (38.5%)
p Value		NS	NS	NS
Size				
≤5 cm	31	22 (71.0%)	12 (38.7%)	15 (48.4%)
>5 cm	15	9 (60.0%)	6 (42.9%)	5 (33.3%)
p Value		NS	NS	NS
Differentiation				
Well/moderate	26	21 (80.8%)	12 (46.2%)	12 (46.2%)
Poor	17	8 (47.1%)	5 (31.3%)	6 (35.3%)
Mucoid	3	2 (66.7%)	2 (66.7%)	2 (66.7%)
p Value		NS	NS	NS
Metastasis				
Present	23	18 (78.3%)	9 (40.9%)	10 (43.5%)
Absent	23	13 (56.5%)	9 (39.1%)	10 (45.5%)
p Value		NS	NS	NS
Serosal invasion				
Present	26	18 (69.2%)	8 (32.0%)	12 (46.2%)
Absent	20	13 (65.0%)	10 (50.0%)	9 (45.0%)
p Value		NS	NS	NS
Clinical stage				
I–II	26	16 (61.5%)	10 (40.0%)	10 (38.5%)
III–IV	20	15 (75.0%)	8 (40.0%)	11 (55.0%)
p Value		NS	NS	NS
Types of carcinoma				
Intestinal	21	15 (71.4%)	6 (30.0%)	9 (42.9%)
Diffuse	17	11 (64.7%)	5 (29.4%)	6 (35.3%)
Mixed	8	5 (62.5%)	7 (87.5%)	5 (62.5%)
p Value		NS	<0.05*	NS

The MUC5AC group contained 45 patients (34 male, 11 female); *intestinal type *v* mixed type group, and diffuse type *v* mixed type group.
NS, not significant.

Lee[10] found that MUC1 positive Korean patients suffering from gastric carcinoma showed significantly poorer survival than those negative for MUC1. Utsunomiya *et al* and Baldus and colleagues[22 23] also found that MUC1 expression was associated with a poor outcome, irrespective of its glycosylation status, as assessed by the use of monoclonal antibodies recognising only core peptides. These conflicting results may reflect different peptide antibody specificity or indicate that specific glycoforms are more reliable as prognostic determinants. In support of this last interpretation, Baldus and colleagues[24] found that MUC1-TF immunoreactivity, which monitors one of the glycoforms of MUC1, was highest in a subgroup of patients with stage I gastric carcinoma. Association with a good prognosis, as in our present study, is consistent with the observation that increased MUC1 mucin is a marker of differentiation in gastric carcinoma,[25] and with the demonstration that both T and B cells of the immune system are activated by MUC1 peptide epitopes. Furthermore, immune responses directed to cryptic epitopes present in tumour associated MUC1 and masked in normal MUC1 mucin have been demonstrated in patients with carcinoma. However, our results and those of previous studies point to a need for greater precision in research linking specific MUC1 epitopes to clinical outcome analyses.

The relation between MUC1 gene expression, clinicopathological features, and the prognosis of patients suffering from breast cancer, pancreatic cancer, and colon cancers has been studied by several groups.[2 26–28] However, there were conflicting results regarding the prognostic value of MUC1, in part because of the use of different antibodies recognising different glycoforms or different core peptides of MUC1 mucin, both of which can be modified during carcinogenesis.[29] The success of future vaccine treatments—for example—may rest upon the identification of specific molecular targets of mucins that mediate tumour invasion.

> "Association with a good prognosis, as in our present study, is consistent with the observation that increased MUC1 mucin is a marker of differentiation in gastric carcinoma, and with the demonstration that both T and B cells of the immune system are activated by MUC1 peptide epitopes"

To our knowledge, our present study is the first attempt to link MUC3 expression to clinicopathological features of gastric tumours. Tumours that expressed MUC3 were associated with more severe disease (stages III–IV and metastasis). Specifically, patients with MUC3 immunoreactivity in their tumours had a higher probability of lymph node metastasis ($p < 0.01$), serosal invasion ($p < 0.05$), and clinical stages III–IV ($p < 0.05$). Similar to many other membrane tethered mucins (MUC4, MUC12, MUC13, and MUC17),[30–33] MUC3 and its rodent orthologue contain EGF-like domains near the C-terminus.[34–36] This specific structural arrangement may be associated with signal transduction and accelerated cell growth, based on studies of rodent Muc4 by Carraway *et al*.[37] These last investigators have shown that there is an interaction between one of the EGF-like motifs of the rMuc4 molecule and the c-erbB-2 molecule on epithelial cell membranes.[9] The c-erbB-2 oncogene belongs to a family of tyrosine kinase growth factor receptors, is present in human

Take home messages

- Membrane bound mucins MUC1 and MUC3 appear to be associated with the development of gastric carcinoma
- Patients who maintained a high anti-MUC1 immunoreactivity had a better prognosis, and those with an increase in anti-MUC3 immunoreactivity had a worse prognosis, as assessed by tumour size, serosal invasion, and metastasis
- There was no correlation between the secretory mucins—MUC2, MUC5AC, and MUC6—and clinical prognosis

gastric cancer, and its overexpression and amplification in gastric cancer is associated with a worse prognosis.[38 39]

We found no relation between MUC2, MUC5AC, or MUC6 immunoreactivity and clinicopathological features. However, there have been some conflicting reports because Utsunomiya and colleagues found that the MUC2 antigen was a prognostic factor associated with a favourable outcome in patients with gastric carcinoma.[23] In contrast, Sakamoto reported that MUC2, as detected by the same antibody (MRP), was related to the histological pattern of the tumour, but did not correlate with clinical outcome.[40] Pinto-De-Sousa *et al* also reported that MUC2, MUC5AC, and MUC6 mucin expression is not associated with the clinicobiological behaviour of the tumours, but is associated with tumour type and location.[41] Thus, although gastric tumours produce secretory mucins, the evidence to date linking this class of mucins to prognosis is weak or contradictory.

In summary, our findings suggest that a focus on the role of membrane mucins, particularly MUC1 and MUC3, rather than secretory mucins, may provide better insight into cancer development and/or prognosis in gastric carcinoma.

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