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Prognostic significance of combined expression of MUC1 and adhesion molecules in advanced gastric cancer

Tetsuro Ohno, Ryuusuke Aihara, Yoichi Kamiyama, Erito Mochiki, Takayuki Asao, Hiroyuki Kuwano*

Department of General Surgical Science, Gunma University Graduate School of Medicine, 3-39-22 Showa-machi, Maebashi, Gunma 371-8511, Japan

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ABSTRACT

The objective of the present study was to evaluate the combination of MUC1 and the status of adhesion molecules in advanced gastric cancers as a possible predictor of patient survival. Two hundred and two paraffin-embedded specimens of gastric carcinoma were examined by immunohistochemical staining using monoclonal antibodies against MUC1 mucin, E-cadherin and β-catenin. The expression of MUC1 was considered positive if at least 10% of the neoplastic cells were stained. E-cadherin and β-catenin were classified into four groups. Only a membranous pattern, which was stained as strongly as normal epithelial cells, was judged as normal. The absent pattern (loss of staining), cytoplasmic pattern (cytoplasmic staining with loss of membranous expression), and heterogeneous pattern (cytoplasmic staining with preservation of membranous expression) were considered abnormal. There was a significant relationship between MUC1-positive expression and abnormal expression of E-cadherin ($P = 0.017$). The cancer with abnormal E-cadherin expression or MUC1-positive expression increased, indicating that the cancer invasion was deep. Survival analysis of the outcome revealed that the survival time for those with abnormal E-cadherin/MUC1-positive expression was shorter than for those with other expression patterns. Multivariate analysis revealed that patients with abnormal E-cadherin/MUC1-positive expression had a poorer prognosis with significance ($P < 0.0001$). In conclusion, abnormal E-cadherin/MUC1-positive expression pattern in advanced gastric cancer is an independent unfavorable prognostic marker.

1. Introduction

Cell–cell adhesion is critical to the establishment and maintenance of normal tissue architecture. Epithelial cadherin (E-cadherin), which is a calcium-dependent cell adhesion molecule, plays a key role in cell–cell epithelial adhesion and epithelial tissue integrity [1]. The intracellular domain of E-cadherin is found in a complex with other submembranous cytosolic proteins (α-catenin and β-catenin), and these catenins mediate the connection of E-cadherin to actin filaments [2]. The catenins are essential for the function of E-cadherin [3]. Although the invasion and metastasis of cancers are complex processes, the disruption of the cadherin-mediated cell–cell adhesion system plays a critical role [2,4].

Mucins are high-molecular-weight glycoproteins that contain oligosaccharides [5] and constitute the major

* *Corresponding author*: Tel.: +81 027 220 8224; fax: +81 027 220 8230.
E-mail address: tetsuro@med.gunma-u.ac.jp (T. Ohno).
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components of the mucus that protects the gastric epithelium from chemical and mechanical aggression [6]. Several mucin genes identified to code for mucin proteins (apomucins) are designated as MUC1, MUC2, MUC3, MUC4, MUC5AC, MUC5B, MUC6, MUC7, MUC8, MUC9, MUC11, MUC12, MUC13, MUC16, MUC19, and MUC20 [7–11]. The normal human stomach expresses MUC1, MUC5AC, and MUC6. MUC1 is widely expressed in mucous cells of the surface epithelium of the antrum and is focally expressed in the oxyntic glands of the body and the pyloric glands of the antrum [12–14]. Alterations in mucin expression take place in gastric carcinomas. It has been reported that MUC1 is frequently over-expressed in carcinomas showing invasive growth and results in poor prognosis in patients with gastric cancer [15] and colorectal cancer [16].

One mechanism by which MUC1 mucin accelerates tumour invasion is via the impairment of E-cadherin [17–19]. MUC1 expression may precede E-cadherin abnormality and contribute to gastric cancer invasion. Several investigators have reported that the immunoreactivity of mucin antigens or E-cadherin in resected carcinoma specimens correlates with many clinicopathological features and patient survival [15,20,21]. However, few studies have evaluated the combined expression of an adhesion molecule and a mucin antigen [22–25]. There is no report about the combined expression of an adhesion molecule and a mucin with exclusive regard to advanced gastric cancer.

The objective of the present study was to investigate whether the expression of MUC1 and that of an adhesion molecule are related and to evaluate the combination of MUC1 and the status of E-cadherin in advanced gastric cancers as a possible predictor of patient survival.

2. Materials and methods

2.1. Histology

This study was based on 202 surgically resected advanced gastric carcinomas (83 differentiated and 119 undifferentiated carcinomas). All of the cases were documented at the Department of General Surgical Science, Gunma University Graduate School of Medicine, Japan, from January 1993 to December 2000. These groups comprised 142 men and 60 women aged 63.0 ± 11.3 (mean ± SD). Pathologic diagnoses and classifications, such as tumour size, depth of invasion, lymphatic and blood vessel permeation, and lymph node metastasis, were used according to the rules of the Japanese Research Society for Gastric Cancer [26]. In addition tumours were classified into two main groups, differentiated and undifferentiated types, defined by the Japanese Research Society for Gastric Cancer [27].

All specimens were fixed in 10% buffered formalin. All lesions were cut into 3–5 mm wide serial step slices and embedded in paraffin. Tissue sections were examined after staining with haematoxylin and eosin (HE). The site of deepest tumour invasion was selected for microscopic examination. Each of these sections was subdivided into serial sections (3 μm thick). One subsection was then used for HE staining, while the others were used for immunohistochemistry.

2.2. Immunohistochemistry

Sections from one or two representative paraffin blocks were stained with the following: (1) MUC1 (Ma552, mouse monoclonal, 1:200; Novocastra); (2) E-cadherin (HECD-1, mouse monoclonal, 1:500; Takara) and (3) β-catenin (17C2, mouse monoclonal, 1:200; Novocastra). Sections were deparaffinized in xylene and hydrated through a graded series of ethanol. Antigens were retrieved by microwaving sections on slides in a 0.01 M citrate buffer, pH 6.0, for 20 min. After the sections were rinsed in phosphate-buffered saline (PBS), endogenous peroxidase was blocked with 3% H_2O_2 in absolute 100% methanol for 30 min. After being rinsed in PBS, the sections were blocked with normal rabbit serum for 30 min and then incubated in humid chambers with one of the primary monoclonal antibodies: MUC1, E-cadherin, or β-catenin overnight at 4 °C. After the sections were rinsed in PBS, the streptavidin–biotin method with Histrofine SAB-PO (mouse) Kits (Nichirei Corp., Tokyo) was used. The sections were incubated for 20 min at room temperature with biotinylated rabbit anti-mouse IgG, A, and M (Nichirei Corp., 10 μg/mL) for 20 min, followed by three washes in PBS. The slides were incubated with peroxidase-conjugated streptavidin (100 μg/mL) for 10 min at room temperature, followed by three washes in PBS. The bound antibody complex was visualized by a reaction in 0.01% H_2O_2 and 0.05% DAB (3,30-diaminobenzidine) for 3 min. A light counterstaining with Mayer's hematoxylin was undertaken. We used non-neoplastic gastric mucosa as an inner control of mucin immunohistochemical staining.

Immunostaining was scored by one observer with no previous knowledge of the clinicopathological details. Staining was evaluated in all layers of carcinomas. The expression of MUC1 was considered positive if at least 10% of the neoplastic cells were stained [12]. The expression of E-cadherin and β-catenin in malignant cells was compared with that of normal epithelial cells. In addition, the staining pattern of E-cadherin and β-catenin was classified into four groups, as reported previously [27]. Only a membranous pattern, which was stained as strongly as normal epithelial cells, was judged as normal. In contrast, the absent pattern (loss of staining), cytoplasmic pattern (cytoplasmic staining with loss of membranous expression), and heterogeneous pattern (cytoplasmic staining with preservation of membranous expression) were considered abnormal. In the case of mixed patterns in some sections, the classification was determined by the dominant pattern.

2.3. Statistical analysis

All statistical analyses were done using the StatView® software (version 5.0; Abacus Concepts Inc., Berkeley, CA). The distributions of parameters in antigen expression were compared using the χ^2 test and the Mann–Whitney *U* test. The student's *t*-test was used to determine group differences. Survival analyses were performed with the Kaplan–Meier method, and differences between the survival curves were tested using the log-rank test. Multivariate survival analysis

was performed using the Cox proportional hazards model. Statistically significant results were determined as a P value of less than 0.05.

3. Results

3.1. *Expression of MUC1, E-cadherin, and β-catenin in carcinoma tissue*

A total of 202 gastric carcinomas were examined immunohistochemically using antibodies Ma552, HECD-1, and 17C2 to detect MUC1, E-cadherin, and β-catenin, respectively. Positive expression of MUC1 was observed in 45% of samples. Abnormal expressions of E-cadherin and β-catenin were 70% and 66%, respectively (Fig. 1).

3.2. *MUC1, E-cadherin, and β-catenin expression and clinicopathological features*

Table 1 shows the relationship between clinicopathological features and antigen expression. Gender, lymphatic invasion, and tumour site showed no correlation with antigen expression. The depths of wall penetration and pTNM stage were correlated with all three types of antigen expression. Age correlated positively with MUC1 expression only. Tumour diameter showed a positive correlation with E-cadherin and β-catenin abnormal expression. Tumours with abnormal expression of E-cadherin showed a positive correlation with venous invasion and lymph node metastasis. Tumours with abnormal expression of β-catenin correlated with an undifferentiated tumour type.

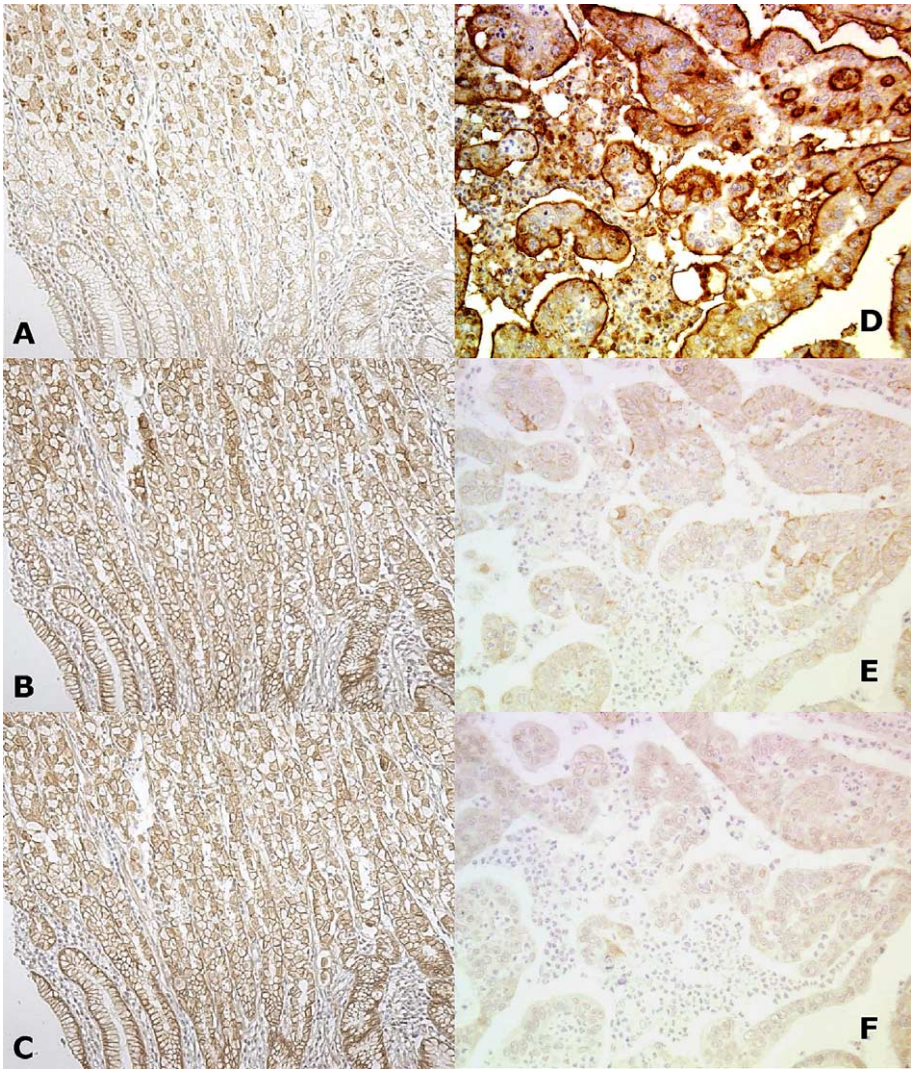


Fig. 1 – Immunohistochemical staining of normal tissue and differentiated-type gastric carcinoma for MUC1, E-cadherin, and β-catenin. (A) MUC1 expression in normal tissue. (B) E-cadherin is expressed in the membrane of normal cells. (C) β-Catenin is expressed in the membrane of normal cells. (D) MUC1 is expressed in the cytoplasm and in the apical membrane in gastric carcinoma. (E) E-cadherin expression is lost (absent pattern) in gastric carcinoma. (F) β-Catenin expression is lost (absent pattern) in gastric carcinoma. (Original magnification, 200×.)

Table 1 – Relationship between clinicopathologic features and antigen expression in 202 advanced gastric cancers

Clinicopathologic feature	MUC1			E-cadherin			β-Catenin		
	Negative	Positive	P[a]	Normal	Abnormal	P[a]	Normal	Abnormal	P[a]
Age (years)[b]	61.5 (11.1)	64.8 (11.4)	**0.040**	61.4 (11.4)	63.7 (11.2)	0.193	63.0 (10.5)	62.9 (11.7)	0.951
Tumor diameter (mm)[b]	58.5 (34.0)	59.1 (27.7)	0.895	50.6 (28.2)	62.2(32.0)	**0.016**	47.1 (21.8)	64.8 (33.8)	**<0.001**
Gender									
Male	77	65	0.591	38	104	0.159	48	94	0.870
Female	35	25		22	38		21	39	
Tumor site									
Upper	29	29	0.484	12	46	0.092	16	42	0.420
Middle	55	37		34	58		35	57	
Lower	28	24		14	38		18	34	
Histological type									
Differentiated	41	42	0.149	25	58	0.914	37	46	**0.009**
Undifferentiated	71	48		35	84		32	87	
Lymphatic invasion									
Absent	8	4	0.420	6	6	0.113	5	7	0.572
Present	104	86		54	136		64	126	
Venous invasion									
Absent	58	35	0.068	35	58	**0.023**	34	59	0.506
Present	54	55		25	84		35	74	
Lymph node metastases									
Absent	40	21	0.057	24	37	**0.049**	24	37	0.307
Present	72	69		36	105		45	96	
Depth of wall penetration									
MP	33	15	**0.048**	22	26	**0.006**	24	24	**<0.001**
SS	43	38		22	59		30	51	
SE	31	31		15	47		14	48	
SI	5	6		1	10		1	10	
pTNM stage									
Ib	31	19	**0.024**	21	29	**0.002**	21	29	**0.008**
II	36	17		20	33		21	32	
IIIA	21	28		9	40		19	30	
IIIB	12	9		5	16		4	17	
IV	12	17		4	25		4	25	

a Mann–Whitney *U* test (depth of wall penetration and pTNM stage), Student's test (age and tumor diameter), χ^2 test (other data).
b Values are mean (s.d.).

3.3. *Relationship between MUC1 and E-cadherin and β-catenin expression*

MUC1-positive expression showed a significant correlation with abnormal E-cadherin expression ($P = 0.017$). However, MUC1-positive expression did not correlate with abnormal β-catenin expression ($P = 0.157$) (Table 2).

Table 2 – Correlation between MUC1 and adhesion molecules' expression

	MUC1 expression		P[a]
	Negative	Positive	
E-cadherin			
Normal	41	19	**0.017**
Abnormal	71	71	
β-Catenin			
Normal	43	26	0.157
Abnormal	69	64	

a χ^2 test.

3.4. *Analysis of MUC1 and E-cadherin status in different stages in two histological tumour types*

Since MUC1 expression correlated with abnormal expression of E-cadherin, the combined status of E-cadherin and MUC1 was assessed at different tumour stages (MP, SS, SE, and SI) and in two histological types of cancer (differentiated and undifferentiated) (Fig. 2). The 202 patients were divided into 4 groups as follows: (1) normal E-cadherin/MUC1-negative ($N = 41$); (2) normal E-cadherin/MUC1-positive ($N = 19$); (3) abnormal E-cadherin/MUC1-negative ($N = 71$) and (4) abnormal E-cadherin/MUC1-positive ($N = 71$). In differentiated-type cancers, normal E-cadherin/MUC1-negative status was detected in 8 patients (29% of differentiated-type tumours), 7 (19%), 1 (6%), and 1 (50%) at MP, SS, SE, and SI, respectively.

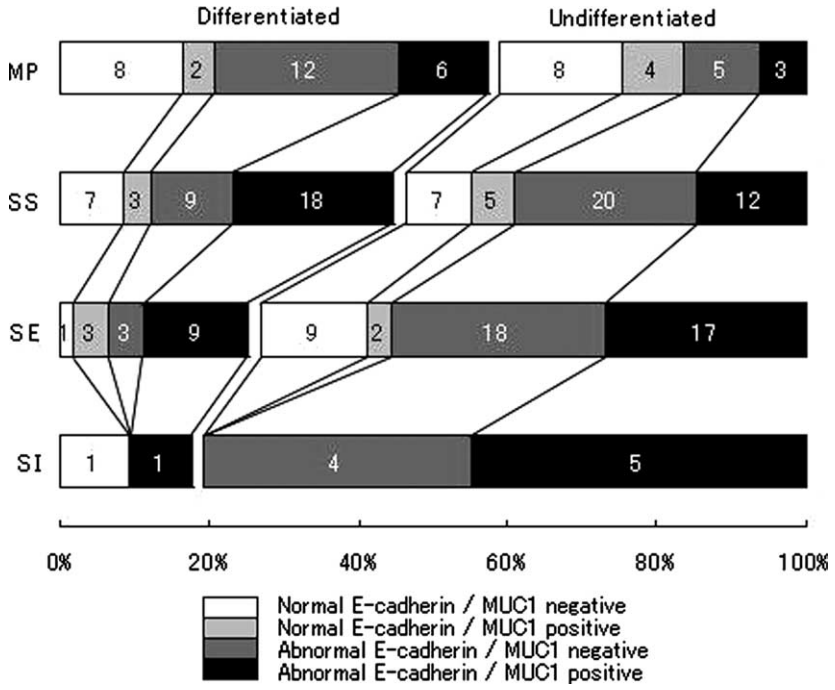


Fig. 2 – Relationship between the expression of E-cadherin and MUC1 mucin at different tumour stages.

In contrast, abnormal E-cadherin/MUC1-positive status was detected in 6 patients (21%), 18 (49%), 9 (56%), and 1 (50%) at MP, SS, SE, and SI, respectively. In undifferentiated-type cancers, normal E-cadherin/MUC1-negative status was detected in 8 patients (40% of undifferentiated-type tumours), 7 (16%), 9 (20%), and 0 (0%) in each stage. Abnormal E-cadherin/MUC1-positive status was detected in 3 patients (15%), 12 (27%), 17 (37%), and 5 (56%), respectively. Cancers with normal E-cadherin expression were not found in the SI stage in differentiated-type carcinomas.

3.5. *Relationship of survival to E-cadherin/MUC1 status*

The relationship between the combined E-cadherin/MUC1 status and post-operative survival was assessed in the 202 patients (Fig. 3). The patients were divided into 4 groups, as stated above. The 5-year survival rate of each group was 83.6%, 53.6%, 63.3%, and 33.1%. Patients with abnormal E-cadherin/MUC1-positive tumours had a significantly lower survival rate after operation.

3.6. *Multivariate analysis of factors related to survival*

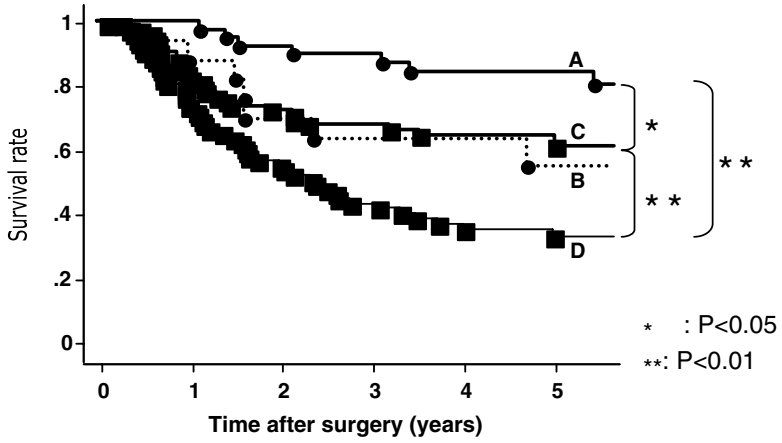
Table 3 shows the results of univariate and multivariate analysis of factors related to survival. The parameters that significantly impacted overall survival were then reviewed by multivariate analysis (Cox proportional hazards model). In the univariate analysis, tumour diameter, histology, venous invasion, and abnormal E-cadherin/MUC1-positive status were significantly related to survival. In the multivariate analysis, all four factors were independently and significantly associated with a poor cancer prognosis. Particularly, patients with abnormal E-cadherin/MUC1-positive expression were found to have a relative risk of death that was 2.8 times higher than that of other patients ($P < 0.0001$).

4. Discussion

In this study, we examined the expressions of MUC1, E-cadherin, β-catenin, and their combinations and investigated the relationship between them and clinicopathological features. In the present study, positive expression of MUC1 was observed in 45% of 202 advanced gastric cancers, and the percentage was lower than that in earlier reports [21,25,28,29]. This difference might be due to the different antibodies used in the studies. Ma552 belongs to a group of antibodies that are reactive to a conformational epitope in the repeat domain and may show a more restricted binding pattern compared to others that have certain sequence specificity and are less affected by the state of O-glycosylation. Alternatively, since we evaluated only advanced gastric cancer, there might be a difference with studies of both early and advanced gastric cancer.

MUC1 expression was related to clinical characteristics, such as the patient's age, depth of wall penetration, and pathological stage. MUC1 was more highly expressed in older patients, and cancers invaded to deeper and more advanced stages. On the other hand, though the expression of MUC1 was not significantly associated with the lymph node metastasis and venous invasion, it still tended towards higher expression in the advanced stages of cancer. These results indicate that MUC1-positive expression alone is an indicator of malignancy of advanced gastric cancer.

In the present study, abnormal expressions of E-cadherin and β-catenin were 70% and 66%, respectively, and consistent with other studies [27,30]. Abnormal expressions of



No. at risk(Survival rate)					
A:Normal E-cadherin/	38	34	33	25	22
MUC1 negative (N=41)	(100%)	(92%)	(89%)	(84%)	(84%)
B:Normal E-cadherin/	15	11	10	9	5
MUC1 positive (N=19)	(94%)	(69%)	(63%)	(63%)	(54%)
C:Abnormal E-cadherin/	59	47	43	30	19
MUC1 negative (N=71)	(83%)	(71%)	(67%)	(63%)	(63%)
D:Abnormal E-cadherin/	52	37	27	20	15
MUC1 positive (N=71)	(76%)	(55%)	(41%)	(35%)	(33%)

Fig. 3 – Kaplan–Meier survival curves of MUC1 and E-cadherin co-expression in gastric carcinoma tissues. Abnormal E-cadherin/MUC1-positive expression was the poorest prognostic factor for survival of gastric carcinoma patients compared to the other expression patterns. The data below shows the number of patients at risk at each time point, and the survival rates are in brackets.

Table 3 – Univariate and multivariate analysis of factors related to survival (Cox proportional hazards model)

Factor	Univariate	Multivariate		
	P value	Hazard ratio	95% CI	*P* value
Age (years)	0.1985			
Gender (female vs. male)	0.6568			
Tumor diameter (mm)	**0.0016**	1.0008	1.001–1.014	**0.0195**
Histology (undifferentiated vs. differentiated)	**0.0066**	2.013	1.286–3.152	**0.0022**
Lymphatic invasion (present vs. absent)	0.0725			
Venous invasion (present vs. absent)	**0.0001**	2.177	1.356–3.494	**0.0013**
Abnormal E-cadherin/MUC1 positive (vs. others)	**<0.0001**	2.833	1.852–4.333	**<0.0001**

CI, confidential interval.

E-cadherin and β-catenin were related to clinical characteristics, such as tumour size, depth of wall penetration, and pathological stage. Abnormal expression of E-cadherin was also related to venous invasion and lymph node metastasis. These results indicate that tumours with abnormal adhesion molecules, which are apt to grow large, invade deeper and progress to more advanced stages.

MUC1 and abnormal expression of adhesion molecules both show malignant characteristics. However, few studies have evaluated the combined expression of an adhesion molecule and a mucin antigen [22–25,27]. In this study, the expression patterns of MUC1 were significantly correlated with E-cadherin expression, but not with β-catenin expression, although there was a tendency (Table 2). Therefore, we have described a possible advanced indicator of malignant potential of gastric cancer using the analysis of E-cadherin status combined with MUC1 expression. Tanaka et al. [27] have evaluated the combined MUC1 and E-cadherin status of both early and advanced gastric cancers. They showed that only early gastric cancer with normal E-cadherin/MUC1-negative expression had no recurrence and a favorable prognosis. In the present study, we evaluated the combined expression of MUC1 and E-cadherin with exclusive regard to advanced gastric cancer. Abnormal E-cadherin expression correlated with MUC1-positive expression. This result supports previous suggestions that MUC1 expressed in cancer cells may disturb cell–cell adhesion mediated by E-cadherin [17–19].

Our analysis revealed that the proportion of tumours with normal E-cadherin/MUC1-negative expression decreased with the depth of wall invasion, and this expression pattern was observed in only one specimen in SI tumours in differentiated carcinoma and was not observed in SI tumours in undifferentiated carcinoma. On the contrary, the percentage of tumours with abnormal E-cadherin/MUC1-positive expression increased with the depth of wall invasion. Moreover, in the SI stage in undifferentiated-type carcinoma, all of the tumours expressed abnormal E-cadherin (Fig. 2). These results suggest that it is difficult for carcinomas to invade deep with normal E-cadherin/MUC1-negative expression, whereas tumours with abnormal E-cadherin/MUC1-positive expression or abnormal E-cadherin expression alone may have the ability to progress to advanced stages. It is thought that the critical role of cancer progression is the reduction of the cell–cell adhesion function by E-cadherin and that MUC1 might disturb this adhesion. However, the total percentage of tumours with abnormal E-cadherin was higher than that of tumours with MUC1-positive expression. There may be tumours that expressed MUC1 in the early stages and then stopped expressing it as the cancer progressed. Further investigations are necessary.

The survival analysis of 202 patients revealed that the survival period for those with abnormal E-cadherin/MUC1-positive expression was shorter than that for those with other expression patterns (Fig. 3). In the multivariate analysis, patients with abnormal E-cadherin/MUC1-positive expression were independently and significantly associated with poor prognosis and found to have a relative risk of death that was 2.8 times higher than that of other patients (Table 3). The multivariate analysis proved that the pattern of abnormal E-cadherin/MUC1-positive expression is a stronger prognostic indicator than existing factors, which demonstrates that this expression pattern is clinically useful.

The immunohistochemical assay used in this study is simple and easily reproduced. It is routinely done in many hospitals. The results obtained by this assay are clinically useful as predicting prognosis and considering adjuvant chemotherapies after surgical resection. In summary, the combined pattern of abnormal E-cadherin/MUC1-positive expression could be an unfavorable marker in a patient with advanced gastric cancer.

Conflict of interest statement

None declared.

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