

ORIGINAL ARTICLE

MUC4 expression is a novel prognostic factor in patients with invasive ductal carcinoma of the pancreas

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Background: Many patients with invasive ductal carcinoma of the pancreas (IDC) have a poor outcome. MUC4 expression has been implicated as a marker for diagnosis and progression of IDC, but there are no studies of the relation between MUC4 expression and patient prognosis in IDC.

Aims: To investigate the prognostic significance of MUC4 expression in IDC.

Methods: The expression profiles of MUC4, ErbB2, p27, and MUC1 were investigated in IDC tissues from 135 patients by means of immunohistochemistry.

Results: MUC4 was expressed in 43 of the 135 patients with IDC (31.9%). The survival of 21 patients with high MUC4 expression (>20% of neoplastic cells stained) was significantly worse than that of the 114 patients with low MUC4 expression (<20% of neoplastic cells stained) ($p = 0.0043$). Univariate analysis showed that high MUC4 expression ($p = 0.0061$), large primary tumour status (>T2) ($p = 0.0436$), distant metastasis ($p = 0.0383$), lymphatic invasion ($p = 0.0243$), and surgical margins ($p = 0.0333$) were significant risk factors affecting the outcome of patients with IDC. Backward stepwise multivariate analysis showed that MUC4 expression ($p = 0.0121$), lymph node metastasis ($p = 0.0245$), and lymphatic invasion ($p = 0.0239$) were significant independent risk factors. ErbB2, p27, and MUC1 were not independent risk factors.

Conclusions: This study shows that MUC4 expression in IDC is a new independent factor for poor prognosis and predicts the outcome of patients with IDC.

Invasive ductal carcinoma of the pancreas (IDC) still has a relatively poor prognosis. In Japan, IDC is the fifth cause of carcinoma related death in men and the sixth cause of carcinoma related death in women.¹ The overall five year survival rate for all patients with or without pancreatectomy after diagnosis is 9.7% in Japan, whereas those patients with a successful resection of IDC at the early stage (stage Ia) have a 39.9% five year survival rate.² Most patients are diagnosed in the advanced stages because of the anatomical location of the pancreas, lack of specific symptoms, infiltration to the surrounding organs, or distant metastasis even from a small primary tumour less than 2 cm in diameter.³

“MUC4 expression is a novel predictor of poor prognosis in patients with intrahepatic cholangiocarcinoma, mass forming type”

Mucins are high molecular weight glycoproteins with oligosaccharides attached to serine or threonine residues of the mucin core protein backbone by *O*-glycosidic linkages, which are produced by various epithelial cells. Core proteins for human mucins (MUC1–9, MUC11–13, and MUC15–17) have been identified.^{4–11} Mucins are categorised into: membrane associated mucins (MUC1, MUC3, MUC4, MUC12, and MUC17), gel forming mucins (MUC2, MUC5AC, MUC5B, and MUC6), and soluble mucin (MUC7).¹² Our previous studies have shown that the expression of MUC1 is related to the invasive proliferation of tumours and poor patient outcome, whereas the expression of MUC2 is related to non-invasive proliferation of tumours and favourable outcome.^{4 13–18} In pancreatic tumours, IDC showed high expression of various glycoforms of MUC1.^{4 19} In contrast, the “dark cell type” and “clear cell type” of intraductal papillary mucinous neoplasms showed different expression patterns of glycosylated MUC1 and MUC2.¹⁹

MUC4 was first reported as tracheobronchial mucin,²⁰ and has been shown to be expressed in various normal human tissues,^{21–24} but not in the normal pancreas.^{25 26} In contrast, MUC4 expression has been detected in IDC.^{25–28} Recently, Swartz *et al* reported that MUC4 expression increases progressively in pancreatic intraepithelial neoplasia (PanIN).^{29 30}

In addition, MUC4 is not expressed in the normal biliary epithelium but it is expressed in cholangiocarcinoma.³¹ We reported for the first time that MUC4 expression is a novel predictor of poor prognosis in patients with intrahepatic cholangiocarcinoma, mass forming type (ICC-MF).³²

To date, no studies are available on the relation between the expression of MUC4 and the outcome of patients with IDC. The aims of our study were to investigate the expression profile of MUC4 in IDC and to evaluate the relation between MUC4 expression and patient outcome.

The transmembrane subunit of MUC4 acts as an intra-membrane ligand for the receptor tyrosine kinase ErbB2.^{33–35} MUC4 plays an important role in cell proliferation and differentiation of epithelial cells³⁴ by inducing specific phosphorylation of ErbB2 and enhancing expression of the cyclin dependent kinase inhibitor p27, which inhibits cell cycle progression through the control of the G1 to S phase.^{36–38} Thus, we also examined the expression of ErbB2 and p27 in IDC and compared it with MUC4 expression. For comparison, we also examined MUC1 expression, which has previously been reported as a useful prognostic factor in various carcinomas.^{18 32 39 40}

Abbreviations: ABC, avidin–biotinylated horseradish peroxidase complex; CI, confidence interval; HR, hazard ratio; ICC-MF, intrahepatic cholangiocarcinoma mass forming type; IDC, invasive ductal carcinoma of the pancreas; PanIN, pancreatic intraepithelial neoplasia; PBS, phosphate buffered saline; SMC, sialomucin complex.

Table 1 Correlation between MUC4, ErbB2, p27, and MUC1 expression in 135 patients with invasive ductal carcinoma of the pancreas

	MUC4 expression				p Value
	-	+	++	+++	
ErbB2 expression					
-	59	14	7	9	0.7595
+	12	5	1	0	
++	16	2	2	1	
+++	5	1	1	0	
p27 expression					
-	52	15	1	5	0.0604
+	9	1	4	1	
++	15	3	4	1	
+++	16	3	2	3	
MUC1 expression					
-	16	3	0	0	0.5999
+	14	2	2	2	
++	27	9	3	2	
+++	35	8	6	6	

The p values were assessed by means of χ^2 test.

MATERIALS AND METHODS

Tissue samples

One hundred and thirty five patients (89 men, 46 women) with surgically resected IDC were retrieved from the files in Kagoshima Medical Association Hospital and Kagoshima University Graduate School of Medical and Dental Sciences (Surgical Oncology and Digestive Surgery), Japan. The mean age of the patients was 65.8 years (range, 30–82). Surgical procedures were as follows: 88 patients were treated by conventional pancreatoduodenectomy, 31 were treated by pylorus preserving pancreatoduodenectomy, 10 were treated by distal pancreatectomy, and six were treated by total pancreatectomy. The 135 cases of IDC were histologically classified according to the TNM postsurgical histopathological classification.⁴¹ The study was approved by the Kagoshima University Graduate School of Medical and Dental Sciences human investigation committee.

The survival period after surgical resection was used as survival time for statistical analysis. The median length of the survival time at the last follow up of the patients was 11.0 months (range, 0–155). Seven patients died in the perioperative period. Clinical outcome data were available in all 135 patients with IDC, and overall survival was analysed. All the specimens were fixed in formalin, embedded in paraffin wax, and cut into 4 μ m thick sections for immunohistochemistry, in addition to the usual haematoxylin and eosin staining.

Immunohistochemistry

Antibodies

Immunohistochemistry was carried out using the following antibodies. MUC4 was detected by a mouse monoclonal antibody, clone 8G7.³⁰ ErbB2 was detected by rabbit polyclonal antibody (Dako Cytomation, Glostrup, Denmark). p27 was detected by mouse monoclonal antibody NCL-p27 (Novocastra, Newcastle, UK). MUC1 was detected by mouse monoclonal antibody DF3 (mouse IgG; Toray-Fuji Bionics, Tokyo, Japan),

Biotinylated affinity purified horse antimouse IgG, goat antirabbit IgG, and avidin–biotinylated horseradish peroxidase (ABC) complex were purchased from Vector Laboratories (Burlingame, California, USA) as the Vectastain Elite ABC kit.

Staining procedure

Immunohistochemical staining was performed with an immunoperoxidase method using the ABC complex as

described previously.^{14 15 17 32} Each section was dewaxed with xylene. Endogenous peroxidase was blocked by incubating the sections in 0.3% hydrogen peroxidase in absolute methanol at room temperature for 30 minutes. After hydration in decreasing concentrations of ethanol in water, the sections were washed in 0.01M phosphate buffered saline (PBS), pH 7.4. Antigen retrieval was achieved by waterbath pretreatment at 80°C for 20 minutes in 0.01M citrate buffer (pH 6.0) for MUC4, waterbath pretreatment at 98°C for 40 minutes for ErbB2, and autoclave pretreatment at 120°C for five minutes for p27. The sections were washed twice with PBS and 2% horse or goat serum in PBS was applied for 30 minutes at room temperature to prevent non-specific staining. The sections were then incubated with dilutions of the primary antibodies (anti-MUC4, 1/3000; anti-ErbB2, 1/100; anti-p27, 1/40; and anti-MUC1, 1/10) in PBS with 1% bovine serum albumin for 16 hours at 4°C. The sections were washed three times with PBS, incubated with the biotinylated secondary antibodies, and then washed three times with PBS. All sections then received ABC complex for 30 minutes. After washing with PBS three times, the sections were finally reacted with diaminobenzidine substrate for 10 minutes for visualisation, rinsed with tap water, counterstained with haematoxylin, and mounted. Reaction products were not present when non-immune serum or PBS was used instead of the primary antibodies.

Evaluation of the results by scoring

The results of the immunohistochemical staining were evaluated as the percentage of positively stained neoplastic cells. For MUC4, ErbB2, and MUC1, membranous and cytoplasmic immunoreactivity was evaluated. For p27, nuclear immunoreactivity was evaluated. According to our previous studies,^{14 32} the percentages of positively stained neoplastic cells were graded as follows: -, < 5% of neoplastic cells stained; +, > 5% but < 20% of neoplastic cells stained; ++, > 20% but < 50% of neoplastic cells stained; and +++, > 50% of neoplastic cells stained.

In addition, for statistical analysis, the 135 cases were divided into two groups: the low expression group, composed of the - and + cases (under 20% of neoplastic cells stained), and the high expression group, composed of the ++ and +++ cases (more than 20% of neoplastic cells stained).¹⁴

Statistical analysis

Statistical analysis was performed using the Student's *t* test, the χ^2 test, and the Mann-Whitney U test where appropriate. Survival of the patients was compared between the group with high MUC4, ErbB2, p27, or MUC1 expression, and the group with low expression. Univariate and multivariate survival analyses were performed using the Cox proportional hazards regression model. For the multivariate model, we used 0.20 as the cutoff p value to select the analysed factors from the univariate analysis data. Furthermore, backward stepwise multivariate analysis was used to find independent prognostic factors. A value of $p < 0.05$ was considered significant.

RESULTS

MUC4, ErbB2, p27, or MUC1 expression in normal pancreatic tissue

MUC4 and ErbB2 were not expressed in normal pancreatic tissue (fig 1A). p27 was expressed in the nuclei of most normal epithelia. MUC1 was expressed in the cell apices of the centroacinar cells, intercalated ducts, and intralobular ducts and focally in the interlobular ducts, but was not expressed in the main pancreatic ducts, acini, or islets.

Table 2 Clinicopathological features and the expression of MUC4, ErbB2, p27, and MUC1 in 135 patients with invasive ductal carcinoma of the pancreas

Category	N (%)	MUC4			ErbB2			p27			MUC1		
		Low expression*	High expression*	p Value	Low expression	High expression	p Value	Low expression	High expression	p Value	Low expression	High expression	p Value
Age (years)													
≤65	51 (37.8)	43 (84.3)	8 (15.7)	0.9740	41 (80.4)	10 (19.6)	0.8003	31 (60.8)	20 (39.2)	0.4888	16 (31.4)	35 (68.6)	0.6198
>65	84 (62.2)	71 (84.5)	13 (15.5)		66 (78.6)	18 (21.4)		56 (66.7)	28 (33.3)		23 (27.4)	61 (72.6)	
Sex													
Male	89 (65.9)	75 (84.3)	14 (15.7)	0.9379	72 (80.9)	17 (19.1)	0.5134	60 (67.4)	29 (32.6)	0.3158	28 (31.5)	61 (68.5)	0.3591
Female	46 (34.1)	39 (84.8)	7 (15.2)		35 (76.1)	11 (23.9)		27 (58.7)	19 (41.3)		11 (23.9)	35 (76.1)	
Primary tumour													
≤T2	20 (14.8)	19 (95.0)	1 (5.0)	0.1582	17 (85.0)	3 (15.0)	0.4927	16 (80.0)	4 (20.0)	0.1153	6 (30.0)	14 (70.0)	0.9054
>T2	115 (85.2)	95 (82.6)	20 (17.4)		90 (78.3)	25 (21.7)		71 (61.7)	44 (38.3)		33 (28.7)	82 (71.3)	
Lymph node metastasis													
No	59 (43.7)	52 (88.1)	7 (11.9)	0.2971	43 (72.9)	16 (27.1)	0.1073	39 (66.1)	20 (33.9)	0.7230	19 (32.2)	40 (67.8)	0.4541
Yes	76 (56.3)	62 (81.6)	14 (18.4)		64 (84.2)	12 (15.8)		48 (63.2)	28 (36.8)		20 (26.3)	56 (73.7)	
Distant metastasis													
No	128 (94.8)	108 (84.4)	20 (15.6)	0.9242	101 (78.9)	27 (21.1)	0.6653	83 (64.8)	45 (35.2)	0.6785	39 (30.5)	89 (69.5)	0.0833
Yes	7 (5.2)	6 (85.7)	1 (14.3)		6 (85.7)	1 (14.3)		4 (57.1)	3 (42.9)		0 (0.0)	7 (100.0)	
Histological grade													
G1	37 (27.4)	32 (86.5)	5 (13.5)	0.7762	24 (64.9)	13 (35.1)	0.0234	27 (73.0)	10 (27.0)	0.2388	15 (40.5)	22 (59.5)	0.1171
G2	73 (54.1)	62 (84.9)	11 (15.1)		60 (82.2)	13 (17.8)		47 (64.4)	26 (35.6)		16 (21.9)	57 (78.1)	
G3	25 (18.5)	20 (80.0)	5 (20.0)		23 (92.0)	2 (8.0)		13 (52.0)	12 (48.0)		8 (32.0)	17 (68.0)	
Lymphatic invasion													
No	9 (6.7)	9 (100.0)	0 (0.0)	0.1826	6 (66.7)	3 (33.3)	0.3348	8 (88.9)	1 (11.1)	0.1128	4 (44.4)	5 (55.6)	0.2865
Yes	126 (93.3)	105 (83.3)	21 (16.7)		101 (80.2)	25 (19.8)		79 (62.7)	47 (37.3)		35 (27.8)	91 (72.2)	
Venous invasion													
No	35 (25.9)	31 (88.6)	4 (11.4)	0.4338	28 (80.0)	7 (20.0)	0.9001	23 (65.7)	12 (34.3)	0.8553	8 (22.9)	27 (77.1)	0.3603
Yes	100 (74.1)	83 (83.0)	17 (17.0)		79 (79.0)	21 (21.0)		64 (64.0)	36 (36.0)		31 (31.0)	69 (69.0)	
Perineural invasion													
No	13 (9.6)	12 (92.3)	1 (7.7)	0.4106	13 (100.0)	0 (0.0)	0.0524	10 (76.9)	3 (23.1)	0.3228	5 (38.5)	8 (61.5)	0.4231
Yes	122 (90.4)	102 (83.6)	20 (16.4)		94 (77.0)	28 (23.0)		77 (63.1)	45 (36.9)		34 (27.9)	88 (72.1)	
Surgical margins													
Negative	78 (57.8)	66 (84.6)	12 (15.4)	0.9489	62 (79.5)	16 (20.5)	0.9391	52 (66.7)	26 (33.3)	0.5281	22 (28.2)	56 (71.8)	0.8375
Positive	57 (42.2)	48 (84.2)	9 (15.8)		45 (78.9)	12 (21.1)		35 (61.4)	22 (38.6)		17 (29.8)	40 (70.2)	

The p values were assessed by means of the χ^2 test.
 *Low expression, under 20% of neoplastic cells stained; high expression, more than 20% of neoplastic cells stained

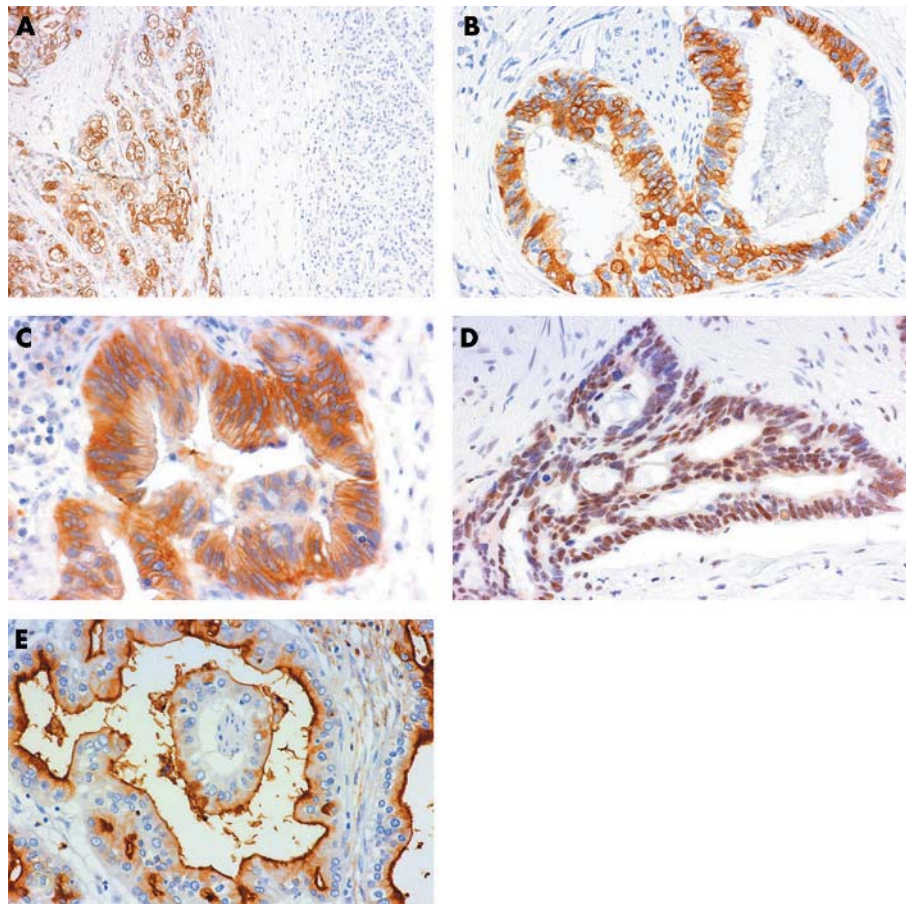


Figure 1 Immunohistochemical staining of (A, B) MUC4, (C) ErbB2, (D) p27, and (E) MUC1 in invasive ductal carcinoma of the pancreas. MUC4 was expressed in the carcinoma tissue (A, left), but not in the normal pancreas tissue (A, right) (original magnification, $\times 40$). MUC4 expression was seen in the cytoplasm and/or membrane of the carcinoma cells (B, original magnification, $\times 160$). ErbB2 was expressed in the membrane and/or cytoplasm of the carcinoma cells (C, original magnification, $\times 160$). p27 expression was seen in the nuclei of the carcinoma cells (D, original magnification, $\times 160$). MUC1 was expressed in the membrane and/or cytoplasm of the carcinoma cells (E, original magnification, $\times 160$).

MUC4, ErbB2, p27, or MUC1 expression profile in IDC

Figure 1 shows representative expression patterns of MUC4, ErbB2, p27, and MUC1 in IDC. Positive staining for MUC4

was seen in the cytoplasm and/or membrane of the carcinoma cells in 43 (31.9%) of 135 IDCs (fig 1A, B).

ErbB2 was expressed at the membrane and/or cytoplasm of the carcinoma cells in 46 (34.1%) of the 135 IDCs (fig 1C), p27 was seen in the nuclei of the carcinoma cells in 62 (45.9%) (fig 1D), and MUC1 was expressed in the membrane and/or cytoplasm of the carcinoma cells in 116 (85.9%) (fig 1E).

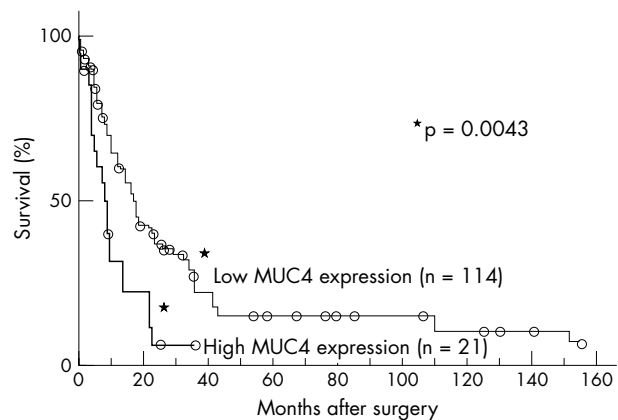


Figure 2 Correlation between MUC4 expression and cumulative survival rate in 135 patients with invasive ductal carcinoma, as determined by the Kaplan–Meier method. The survival of patients with high MUC4 expression was worse than those with low MUC4 expression ($p = 0.0043$). The open circles represent those patients who were alive at the last follow up.

Association between MUC4, ErbB2, p27, and MUC1 expression in IDC

There was no significant association between the expression of MUC4 and the expression of ErbB2, p27, or MUC1 ($p = 0.7595, 0.0604, \text{ and } 0.5999$, respectively; table 1).

Association between expression of MUC4, ErbB2, p27, or MUC1 and clinicopathological features

Table 2 shows the association between the expression of MUC4, ErbB2, p27, or MUC1 and the clinicopathological features. In the 135 patients examined, 21 (15.6%) had high MUC4 expression and 114 (84.4%) had low MUC4 expression. MUC4 expression in IDC was not related to the clinicopathological features listed in table 2. Twenty eight (20.7%) patients had high ErbB2 expression and 107 (79.3%) had low ErbB2 expression. The expression of ErbB2 was related to histological grade ($p = 0.0234$). Neither expression of p27 nor MUC1 was related to the clinicopathological features listed in table 2.

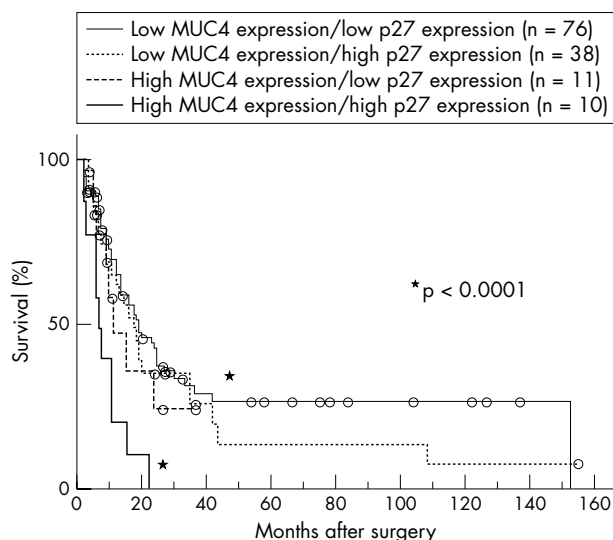


Figure 3 Combined evaluation of MUC4 and p27 expression. Patients with both high MUC4 and p27 expression had the worst outcome. In contrast, patients with both low MUC4 and p27 expression had the best outcome. A significant difference for survival was found between the two groups ($p < 0.0001$). The open circles represent those patients who were alive at the last follow up.

Association between expression of MUC4, ErbB2, p27, MUC1, or histological parameters and cumulative survival rate

Among the 135 patients examined, 87 died during the follow up period. Forty eight patients were alive at the last follow up and the longest survivor was alive at 155 months after surgery. The overall one, three, and five year survival rates of patients with surgical resection were 58.7%, 19.0%, and 11.4%, respectively.

The one, three, and five year survival rates of the high MUC4 expression group were 31.5%, 5.6%, and 0.0%, respectively, whereas those of the low MUC4 expression group were 64.2%, 22.0%, and 13.9%, respectively. The survival of patients with high MUC4 expression was significantly worse than those with low MUC4 expression ($p = 0.0043$; log rank test; fig 2). There were no significant associations between survival and the expression of erbB2, p27, or MUC1 ($p = 0.5400, 0.0726, \text{ and } 0.9730$, respectively; log rank test).

In the combined evaluation of MUC4 and p27 expression, patients with both high MUC4 and p27 expression had a worse outcome than those with both low MUC4 and p27 expression. A significant difference for survival was found between the two groups ($p < 0.0001$; fig 3). There was no significant difference in the combined evaluation of MUC4 and ErbB2 expression, or in that of MUC4 and MUC1 expression ($p = 0.5369 \text{ and } 0.0582$, respectively).

The survival of patients with large primary tumour status ($> T2$), positive distant metastasis, positive lymphatic invasion, or positive surgical margins was significantly worse than those with small primary tumour status ($\leq T2$) ($p = 0.0089$), no distant metastasis ($p = 0.0292$), no lymphatic invasion ($p = 0.0156$), or negative surgical margins ($p = 0.0287$) (log rank test).

Univariate analysis of prognostic factors

Table 3 shows the results of univariate analysis of prognostic factors for IDC. MUC4 expression ($p = 0.0061$; hazard ratio (HR), 2.127; 95% confidence interval (CI), 1.240 to 3.649), large primary tumour status ($> T2$) ($p = 0.0436$; HR, 1.846; 95% CI, 1.018 to 3.350), distant metastasis ($p = 0.0383$; HR,

Table 3 Univariate analysis of prognostic factors

Variable	HR	95% CI	p Value
Age (years)			
≤65 (n = 51)	1		
>65 (n = 84)	1.130	0.737 to 1.735	0.5749
Sex			
Male (n = 89)	1		
Female (n = 46)	1.080	0.675 to 1.729	0.7470
Primary tumour			
≤T2 (n = 20)	1		
>T2 (n = 11)	1.846	1.018 to 3.350	0.0436
Lymph node metastasis			
No (n = 59)	1		
Yes (n = 76)	1.542	0.983 to 2.420	0.0595
Distant metastasis			
No (n = 128)	1		
Yes (n = 7)	2.438	1.049 to 5.665	0.0383
Histological grade			
G1 (n = 37)	1		
G2 (n = 73)	1.123	0.565 to 2.231	0.7401
G3 (n = 25)	1.280	0.676 to 2.425	0.4483
Lymphatic invasion			
No (n = 9)	1		
Yes (n = 126)	3.208	1.163 to 8.846	0.0243
Venous invasion			
No (n = 35)	1		
Yes (n = 100)	1.007	0.641 to 1.584	0.9745
Perineural invasion			
No (n = 13)	1		
Yes (n = 122)	1.520	0.725 to 3.186	0.2673
Surgical margins			
Negative (n = 78)	1		
Positive (n = 57)	1.608	1.038 to 2.490	0.0333
MUC4 expression			
Low (<20%) (n = 114)	1		
High (≥20%) (n = 21)	2.127	1.240 to 3.649	0.0061
ErbB2 expression			
Low (<20%) (n = 107)	1		
High (≥20%) (n = 28)	1.182	0.685 to 2.039	0.5482
p27 expression			
Low (<20%) (n = 87)	1		
High (≥20%) (n = 48)	1.469	0.955 to 2.258	0.0798
MUC1 expression			
Low (<20%) (n = 39)	1		
High (≥20%) (n = 96)	1.001	0.635 to 1.599	0.9735

CI, confidence interval; HR, hazard ratio.

2.438; 95% CI, 1.049 to 5.665), lymphatic invasion ($p = 0.0243$; HR, 3.208; 95% CI, 1.163 to 8.846), and surgical margins ($p = 0.0333$; HR, 1.608; 95% CI, 1.038 to 2.490) were significant poor risk factors affecting the outcome of the patients.

Multivariate analysis of prognostic factors

Table 4 shows the results of multivariate analysis of IDC prognostic factors. Among the seven factors selected from the univariate analysis data, based on 0.20 as the cutoff p value, MUC4 expression ($p = 0.0165$, HR, 1.956; 95% CI, 1.130 to 3.384) was the only significant poor risk factor affecting the outcome of the patients.

Backward stepwise multivariate analysis of prognostic factors

Table 5 shows the results of the backward stepwise multivariate analysis of the seven prognostic factors listed in table 4. MUC4 expression ($p = 0.0121$; HR, 2.005; 95% CI, 1.164 to 3.452), lymph node metastasis ($p = 0.0245$; HR, 1.687; 95% CI, 1.069 to 2.660), and lymphatic invasion ($p = 0.0239$; HR, 3.282; 95% CI, 1.170 to 9.207) were significant independent risk factors.

DISCUSSION

The expression of MUC4 has been studied in IDC tissue samples and cell lines, and its relation to malignant

Table 4 Multivariate analysis of prognostic factors

Variable	HR	95% CI	p Value
Primary tumour			
≤T2 (n=20)	1		
>T2 (n=115)	1.057	0.546 to 2.048	0.8691
Lymph node metastasis			
No (n=59)	1		
Yes (n=76)	1.547	0.955 to 2.507	0.0764
Distant metastasis			
No (n=128)	1		
Yes (n=7)	1.532	0.641 to 3.664	0.3372
Lymphatic invasion			
No (n=9)	1		
Yes (n=126)	2.672	0.889 to 8.034	0.0801
Surgical margins			
Negative (n=78)	1		
Positive (n=57)	1.372	0.880 to 2.140	0.1631
MUC4 expression			
Low (<20%) (n=114)	1		
High (≥20%) (n=21)	1.956	1.130 to 3.384	0.0165
p27 expression			
Low (<20%) (n=87)	1		
High (≥20%) (n=48)	1.280	0.820 to 1.997	0.2775

CI, confidence interval; HR, hazard ratio.

progression has been elucidated.^{25–28} The expression of MUC4 is thought to be a useful indicator of malignancy potential in IDCs,^{28,30} but there have been no studies of the direct association between the expression of MUC4 and the outcome of patients with IDC. We have reported that MUC4 expression is a very useful predictor of poor prognosis in patients with ICC-MF: the survival rate of MUC4 positive patients was significantly worse than MUC4 negative patients.³²

In our present study, we demonstrated for the first time that patients with IDC who have high MUC4 expression had a worse survival rate than those with low MUC4 expression, similar to patients with ICC-MF. Univariate analysis of the prognostic factors in our present study showed that MUC4 expression, primary tumour size, distant metastasis, lymphatic invasion, and surgical margins were significant risk factors affecting the outcome of patients with IDC. Furthermore, MUC4 expression was the only significant prognostic factor for patients with IDC in the multivariate analysis. In the evaluation of independent risk factors using backward stepwise multivariate analysis, MUC4, lymph node metastasis, and lymphatic invasion were significant independent risk factors in patients with IDC. These findings are in line with the progression model for pancreatic adenocarcinoma reported by Swartz *et al*, showing that MUC4 expression increases with increasing grade of PanIN.³⁰

MUC4/sialomucin complex (SMC) is a rat homologue of the human mucin gene MUC4, and its transmembrane subunit acts as an intramembrane ligand for the receptor tyrosine kinase ErbB2 to induce the phosphorylation of the

Tyr-1248 of ErbB2.^{34,36,42} MUC4/SMC leads to the expression of the cell cycle inhibitor p27.³⁴ In addition, MUC4/SMC and neuregulin act synergistically to enhance phosphorylation of both ErbB2 and ErbB3, resulting in the downregulation of p27 and activation of protein kinase B/Akt.^{35,36} It is proposed that complex formation between MUC4/SMC and ErbB2 has an effect on epithelial cell behaviour, as a switch in epithelial differentiation and proliferation.^{35,36} Furthermore, Singh *et al* proposed that MUC4 participates in tumour growth and metastasis by directly altering tumour cell properties and/or via modulating ErbB2 expression.⁴³ However, in the IDCs examined in our present study, ErbB2 expression was not a significant prognostic factor, and neither was combined MUC4 and ErbB2 expression. Nevertheless, high ErbB2 expression was more frequently seen in well differentiated than in poorly differentiated IDCs. This finding is compatible with a report that that well or moderately differentiated colorectal cancers more frequently express ErbB2 proteins than poorly differentiated tumours.⁴⁴

“Patients with both high MUC4 and p27 expression had a significantly worse outcome than those with both low MUC4 and p27 expression, indicating that p27 upregulation is induced by MUC4 expression in invasive ductal carcinoma”

Low expression of p27 is a poor prognostic factor in gastric cancer, colorectal carcinoma, and intrahepatic cholangiocarcinoma.^{45–47} However, in the IDCs examined in our present study, p27 expression was not an independent prognostic factor, although patients with both high MUC4 and p27 expression had a significantly worse outcome than those with both low MUC4 and p27 expression. This result indicates that p27 upregulation is induced by MUC4 expression in IDC. The relation between p27 upregulation and MUC4 expression may be partly explained by the model proposed by Carraway *et al* and Jepson *et al*.^{35,36}

MUC4 extends at least 1.12–2.12 μm above the cell membrane, far above all other membrane associated proteins, such as adhesion molecules.^{43,48} With its rigid and extended structure, MUC4 is thought to be a modulator of cell–cell and cell–extracellular matrix interactions.⁴⁹ Komatsu *et al* showed that SMC disrupts integrin mediated cell adhesion to extracellular matrix proteins.^{49,50} In addition, overexpression of SMC masks the surface antigens on target tumour cells and effectively suppresses tumour cell killing by cytotoxic lymphocytes.⁴⁹ These phenomena may be related to the poor outcome of patients with high MUC4 expression, although MUC4 expression was not related to morphological invasive parameters, such as lymphatic invasion, venous invasion, perineural invasion, and distant lymph node metastasis.

MUC1 is also a transmembrane protein, and MUC1 expression is related to the development of tumours.^{51,52} MUC1 expression enhances tumour detachment from the primary site and accelerates distant metastasis, through binding to E selectin and/or intercellular adhesion molecule 1 of the endothelial cells.^{53–55} In previous studies, MUC1 expression was related to poor outcome in patients with various carcinomas.^{18,32,39,40} In our previous study of IDC,⁵⁵ MUC1 expression was also a poor prognostic factor in advanced cases, although our present study, which includes not only advanced but also early cases, found no relation between the expression of MUC1 and the outcome of patients with IDC.

In ICC-MF, MUC4 expression is a more significant prognostic factor than MUC1 expression in multivariate analysis.³² In ICC-MF, MUC1 was expressed at the luminal surface membrane and/or in the cytoplasm, whereas MUC4

Table 5 Backward stepwise multivariate analysis of prognostic factors

Variable	HR	95% CI	p Value
Lymph node metastasis			
No (n=59)	1		
Yes (n=76)	1.687	1.069 to 2.660	0.0245
Lymphatic invasion			
No (n=9)	1		
Yes (n=126)	3.282	1.170 to 9.207	0.0239
MUC4 expression			
Low (<20%)(n=114)	1		
High (≥20%)(n=21)	2.005	1.164 to 3.452	0.0121

CI, confidence interval; HR, hazard ratio.

Take home messages

- The survival of patients with invasive ductal carcinoma (IDC) of the pancreas with high MUC4 expression (>20% of neoplastic cells stained) was significantly worse than that of those with low MUC4 expression (<20% of neoplastic cells stained)
- Thus, MUC4 expression in IDC is a new independent factor for poor prognosis and predicts the outcome of patients with IDC

expression was seen in the cytoplasm only. In our present study, we confirmed the cytoplasmic expression pattern of MUC4 in IDC also. MUC4 is a membrane mucin, as is MUC1, but MUC4 acts by a different mechanism from that of MUC1. MUC1 acts as a docking protein for signalling molecules, whereas MUC4 acts as a receptor ligand.³⁴ The different expression patterns of MUC1 and MUC4 suggest the possibility of different mechanisms of expression of MUC1 and MUC4, both of which are membrane mucins with cell signalling functions.

The combined evaluation of MUC4 and MUC1 expression also showed no significant difference in survival for patients with IDC in our present study, although there was a significant difference between MUC4 and MUC1 positive patients and MUC4 and MUC1 negative patients in ICC-MF.³²

In conclusion, our results provide new and important information on the importance of MUC4 expression as a useful indicator to predict the outcome of patients with surgically resected IDC. MUC4 has potential as a prognostic marker for clinical management of patients with IDC.

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ECHO

Postmortems on very preterm infants are valuable



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Postmortem examinations on very premature infants are valuable to help doctors to understand and accept the certainty of death in certain circumstances and to help them advise parents, according to a small study. They may also help in future prognoses, and their findings may lead changes in intensive care policies and protocols.

Postmortem examinations on 29 infants of < 28 weeks' gestation who died in intensive care within 28 days after their birth resulted in new diagnoses in no less than 79% and significant change in diagnosis in 28%, when compared with the clinical diagnosis. Injuries caused by treatment were evident in 41% of the group and were considered to be the main cause of death in 4% (four infants). Elsewhere published rates are 4–15%. Some deaths were due to complications of extreme prematurity such as lung disease, but complications of pregnancy or labour also featured.

The examinations were performed by an experienced perinatal pathologist at a major paediatric centre in New Zealand on 29 of 54 infants meeting the criteria who died during January 1995–December 2003.

Extreme prematurity can be given as the cause of death on the death certificate when extremely preterm infants die, but this tells us little and does not advance our knowledge, especially as infants this young are less likely to have postmortem examinations anyway.

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MUC4 expression is a novel prognostic factor in patients with invasive ductal carcinoma of the pancreas

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