ORIGINAL ARTICLE

Mucin expression in pleomorphic adenoma of salivary gland: a potential role for MUC1 as a marker to predict recurrence

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Background: Pleomorphic adenoma of the salivary gland (PA) is essentially a benign neoplasm. However, patients with recurrent PA are difficult to manage. There are rare reports on useful immunohistochemical markers to detect a high risk of recurrence when the primary lesions are resected.
Aims: To find a new marker to predict the recurrence of PA.
Methods: Primary lesions of PA were collected from nine patients showing subsequent recurrence and from 40 patients without recurrence during at least 10 years of follow up of the disease. Paraffin wax embedded tumour samples of the two groups were examined for the expression profiles of MUC1 (differentially glycosylated forms), MUC2, MUC4, MUC5AC, and MUC6 using immunohistochemistry. Several clinicopathological factors were also examined.
Results: In univariate analysis of the factors examined, MUC1/DF3 high expression (more than 30% of the neoplastic cells stained) in the primary lesions was seen more frequently in patients with recurrence (four of nine) than in those without recurrence (three of 40; $p = 0.011$). Larger tumour size (more than 3.0 cm) of the primary PA was also a significant ($p = 0.035$) risk factor for the recurrence of PA. In multivariate analysis, only high expression of MUC1/DF3 was found to be a significant independent risk factor for the recurrence of PA ($p = 0.021$).
Conclusions: Expression of MUC1/DF3 in PA is a useful marker to predict its recurrence. Those patients with PA showing positive MUC1/DF3 expression should be followed up carefully.

Pleomorphic adenoma (PA) is the most common tumour of the salivary gland. Although this tumour is originally benign, the rate of recurrence is relatively high (2.5–32.5%).[1–5] The frequency of recurrence is lower in most modern surgical series, although once recurrence occurs PA may have a high rate of re-recurrence.[6] Recurrent PAs are often multinodular and frequently lack surrounding capsule, so that patients with recurrent PA of the salivary gland are difficult to manage.[5 7 8] The prediction and prevention of recurrence are important for the treatment of PA.

An increased risk of local recurrence after primary surgery for PA has been suggested to be associated with the occurrence of pseudopodia,[2] younger age of the patient,[9–11] the stroma rich variant,[11] and incomplete surgical excision.[8 9] Incomplete surgical excision is believed to be the most essential factor, although in our experience, a few patients with PA treated by curative resection showed subsequent recurrence, and some with PA carrying residual tumours showed no recurrence (unpublished data; 26 cases in our files). Recently, it has also been reported that the stroma rich variant,[12] intraoperative tumour spill,[1 2 12 13] and inadequate resection[4] may not be associated with an increased risk of recurrence. Consequently, the mechanism of PA recurrence is unclear. Although some recent immunohistochemical studies have revealed the intrinsic biological characters of this tumour, such as cell proliferation activity and overexpression of the progesterone receptor, the oestrogen receptor, and p53,[14–16] there were few useful markers that enabled us to predict subsequent recurrence at the point of initial surgery of PA.

Mucins are high molecular weight glycoproteins with oligosaccharides attached to serine or threonine residues of the mucin core protein backbone by *O*-glycosidic linkages. During the past few years, core proteins for human mucins (MUC1–9, MUC11–13, and MUC15–17) have been identified and named chronologically.[17 18] Of the mucins identified, MUC1 is a membrane bound mucin detected in most epithelial tissues.[17] MUC2 is an intestinal-type secretory mucin and is expressed mainly in goblet cells of the intestine.[19] MUC4 was first reported as tracheobronchial mucin,[20] and is a membrane mucin.[21] Similar to MUC2, MUC5AC and MUC6 are secretory mucins and are expressed mainly in the gastric surface mucous cells and pyloric glands, respectively.[22]

> ''Recurrent pleomorphic adenomas are often multinodular and frequently lack surrounding capsule, so that patients with recurrent PA of the salivary gland are difficult to manage''

Our immunohistochemical studies for mucin expression in various human tumours have shown that the expression of MUC1 mucin is associated with invasive growth of the tumours and poor outcome of the patients, whereas the expression of MUC2 mucin is associated with non-invasive growth of the tumours and a favourable outcome of the patients.[23–29] MUC4 has already been identified in normal salivary glands,[30] and has been reported to be expressed in pancreatic adenocarcinoma.[31] We also recently reported that MUC5AC was highly expressed in both aggressive and indolent tumours of the pancreas (invasive ductal carcinoma

Abbreviations: ABC, avidin–biotin complex; CI, confidence interval; MAb, monoclonal antibody; PA, pleomorphic adenoma; PBS, phosphate buffered saline; OR, odds ratio

and intraductal papillary mucinous neoplasm of the pancreas),[32] and that MUC2 and MUC6 were highly expressed in mucinous carcinoma of the breast with less aggressive biological behaviour.[33] Thus, it is of interest to investigate the relation between the expression of these mucins and recurrence in PA.

In our present study, to find a useful marker to predict the recurrence of PA, we examined the expression profiles of different glycoforms of MUC1 mucins using five monoclonal antibodies (MAbs) of differing carbohydrate specificities (NCL-MUC-1-CORE, DF3, MY.1E12, NCL-MUC-1, and HMFG-1), in addition to antibodies to MUC2, MUC4, MUC5AC, and MUC6 in the primary lesions of PA; we compared these expression profiles between a group of patients in whom post-surgical recurrence occurred and a group in whom no recurrence was seen using immunohistochemistry. Basic clinicopathological factors were also compared between the two groups.

MATERIALS AND METHODS

Tissue samples

Primary lesions from nine patients with PA (parotid gland, seven; submandibular gland, one; palate, one) showing subsequent recurrence (designated as ''recurrence group'') were retrieved from the files of the first department of oral and maxillofacial surgery, Kagoshima University dental school, and department of pathology, Kagoshima City Hospital, Kagoshima, Japan. Twenty three patients who had recurrent PA lesions were found in our files, but the primary lesion specimens were available in only nine. The average interval between resection of the primary lesion and the initial recurrence was 6.4 years (range, 3–11) in the nine patients. The rate of re-recurrence was 22% (two of nine) in the recurrence group. For comparison, primary lesions from 40 patients with PA (parotid gland, 17; submandibular gland, seven; palate, 13; other oral mucosa, three) showing no recurrence during at least 10 years of follow up (designated as ''non-recurrence group'') were retrieved from the same files. The primary lesions showing subsequent recurrence were resected between 1975 and 1994. The primary lesions showing no recurrence were resected between 1985 and 1992. Our study was ethically approved by the Kagoshima University Faculty of Medicine human investigation committee.

All the specimens were fixed in 10% formalin, embedded in paraffin wax, and cut into 4 μm thick serial sections for immunohistochemistry, in addition to the usual haematoxylin and eosin staining.

Immunohistochemistry

Antibodies

Table 1 details the antibodies used for immunohistochemistry. For MUC1 expression, different glycoforms of MUC1 were examined by five MAbs, namely: NCL-MUC-1-CORE, DF3, MY.1E12, NCL-MUC-1, and HMFG-1. NCL-MUC-1-CORE recognises MUC1 mucin core peptide (GVTSAPDTRPAP)[34] (clone Ma552; Novocastra Laboratories Ltd, Newcastle, UK). DF3 identifies MUC1 core peptide (APDTRPAP).[34] Although the binding of DF3 to protein has been reported to be enhanced by the presence of carbohydrates,[35] a recent study found that DF3 does not recognise a carbohydrate containing epitope.[34] MY.1E12 is specific for sialylated MUC1 mucin.[36] NCL-MUC-1 recognises a carbohydrate epitope of MUC1 (exact epitope unknown)[37] (clone Ma695; Novocastra Laboratories). The binding of HMFG-1 (kindly provided by Dr J Taylor-Papadimitriou, Imperial Cancer Research Fund Laboratories, London, UK) to core protein epitopes (APDTR)[34] is influenced by the carbohydrate chains and by sialic acid, although it detects fully glycosylated MUC1 mucin.[38] For simple representation, ''MUC1/CORE, MUC1/DF3, MUC1/MY.1E12, MUC1/glycoprotein, and MUC1/HMFG-1'' were used for the MUC1 mucin antigens detected by MAbs NCL-MUC-1-CORE, DF3, MY.1E12, NCL-MUC-1, and HMFG-1, respectively.

Table 1 Monoclonal antibodies used

Antigen	Antibodies
MUC1 mucin	
MUC1/CORE (core peptide of MUC1 mucin)	NCL-MUC-1-CORE (clone Ma552)
MUC1/DF3 (core peptide of MUC1 mucin)	DF3
MUC1/MY.1E12 (sialylated MUC1 mucin)	MY.1E12
MUC1/Glycoprotein (sialylated MUC1 mucin)	NCL-MUC-1 (clone Ma695)
MUC1/HMFG-1 (fully glycosylated MUC1 mucin)	HMFG-1
MUC2 mucin	
MUC2 (core peptide of MUC2 mucin)	NCL-MUC-2 (clone Ccp58)
MUC4 mucin	
MUC4 (core peptide of MUC4 mucin)	Clone 8G7
MUC5AC mucin	
MUC5AC (core peptide of MUC5AC mucin)	NCL-MUC-5AC (clone CLH2)
MUC6 mucin	
MUC6 (core peptide of MUC6 mucin)	NCL-MUC-6 (clone CLH5)

NCL-MUC-1-CORE, mouse IgG, hybridoma culture supernatant (Novocastra Laboratories, Newcastle, UK); DF3, mouse IgG, ascites, and Centocor CA15-3 (Toray-Fuji Bionics, Tokyo, Japan); MY.1E12, mouse IgG, ascites (provided by Dr T Irimura, University of Tokyo, Tokyo, Japan); NCL-MUC-1, mouse IgG, hybridoma culture supernatant (Novocastra Laboratories); HMFG-1, mouse IgG, hybridoma culture supernatant (provided by Dr J Taylor-Papadimitriou, Imperial Cancer Research Fund, London, UK); NCL-MUC-2, anti-MUC5AC and anti-MUC6, mouse IgG, hybridoma culture supernatant (Novocastra Laboratories); clone 8G7, mouse IgG (generated by S K Batra, Department of Biochemistry and Molecular Biology, Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, USA).

MUC2 was detected by MAb NCL-MUC-2 (clone Ccp58; Novocastra Laboratories). MUC4 was detected by mouse MAb clone 8G7.[31 39] MUC5AC and MUC6 were detected by MAb NCL-MUC-5AC (clone CLH2) and MAb NCL-MUC-6 (clone CLH5), respectively (Novocastra Laboratories).

Biotinylated, affinity purified horse antimouse 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 were carried out using an immunoperoxidase method and the ABC complex as described previously.[25–27 33] Briefly, each section was dewaxed with xylene. Endogenous peroxidase was blocked by incubating the sections in 0.3% hydrogen peroxide in absolute methanol at room temperature for 30 minutes. After hydration in decreasing concentrations of ethanol in water, the sections were washed in 10mM phosphate buffered saline (PBS), pH 7.4. Next, 2% horse serum in PBS was applied for 30 minutes at room temperature to prevent non-specific staining. The sections were incubated with dilutions of the primary antibodies (NCL-MUC-1-CORE, 1/100; DF3, 1/10; MY.1E12, 1/200; NCL-MUC-1, 1/100; HMFG-1, 1/100; NCL-MUC-2, 1/600; clone 8G7, 1/3000; NCL-MUC-5AC, 1/100; and NCL-MUC-6, 1/100) in PBS with 1% bovine serum albumin for 16 hours at 4℃. The sections were washed three times with PBS, incubated with the biotinylated secondary antibody, and then washed three times with PBS. All the sections were then incubated with the ABC complex for 30 minutes. After washing with PBS three times, the sections were finally

Table 2 Clinicopathological factors and mucin expression in the primary lesions for each patient in the recurrence group

Case	Age (years)	Sex	Site	Size (cm)*	Margin†	Capsular invasion	Tumour variant‡	Mucin expression								Recurrence
								MUC1								
								CORE	DF3	MY.IE12	Glycoprotein	HMFG-1	MUC2	MUC4	MUC5AC	MUC6	
1	35	F	Palate	4.5	+	+	A	–	–	–	–	–	–	–	–	–	1st (3y), 2nd (5y) 3rd (9y), 4th (12y)
2	32	M	Parotid	2.2	+	–	A	–	–	–	–	–	–	–	–	–	1st (7y)
3	27	M	Parotid	4.0	–	–	A	–	–	–	+	–	+	–	–	+	1st (4y)
4	62	F	Parotid	2.0	+	+	A	–	+	–	+	–	–	–	–	+	1st (5y), 2nd (13y)
5	60	F	Parotid	4.5	+	–	C	–	++	–	–	+	–	–	–	–	1st (11y)
6	53	M	Parotid	3.0	–	+	A	–	+	+	–	–	–	–	+	+	1st (8y)
7	50	M	Parotid	3.0	–	–	A	+	++	++	++	–	–	–	–	+	1st (4y)
8	32	F	SM	4.0	+	+	B	++	++	++	++	++	–	–	–	++	1st (6y)
9	38	F	Parotid	1.5	–	+	B	+	++	+	+	–	–	–	–	–	1st (10y)

*Maximum diameter; †+, incomplete surgical resection; –, complete surgical resection microscopically; ‡A, stroma rich variant; B, intermediate, C, stroma poor variant.
The positively stained neoplastic cells were graded as follows: –, less than 5% of neoplastic cells stained; +, more than 5% but less than 30% of neoplastic cells stained; ++, more than 30% of neoplastic cells stained.
SM, submandibular.

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 percentages of positively stained neoplastic cells were graded as follows: −, less than 5% of neoplastic cells stained; +, more than 5% but less than 30% of neoplastic cells stained; ++, more than 30% of neoplastic cells stained. In addition, for statistical analysis, the cases were divided into two groups: the low expression group, composed of the − and + cases (less than 30 % of neoplastic cells stained), and the high expression group, composed of the ++ cases (more than 30% of the neoplastic cells stained).

Statistical analysis

Both the clinicopathological factors and the ratio of the numbers of patients classified into the high and low expression groups for each mucin were compared between the recurrence group and non-recurrence group, with significant ($p < 0.05$) differences determined by the χ^2 test or Fisher's exact test. Univariate logistic regression analysis was then conducted to evaluate the risk on the association of recurrence as estimated by the odds ratio (OR) and 95% confidence intervals (95% CI). For the multivariate model, to evaluate the independent risk factors on the association of recurrence, we used 0.20 as the cutoff p value to select the analysed factors from the univariate analysis data. Because preliminary analysis showed relative risks for recurrence associated with patient's age younger than 35 years and with tumour size larger than 3.0 cm, those categories were grouped and served as the reference for the age variant and for the size variant. A probability of $p < 0.05$ was considered significant.

RESULTS

Table 2 summarises the clinicopathological features and mucin expression profiles in the primary lesions of the nine patients in the recurrence group. Table 3 summarises these data in the 40 patients in the non-recurrence group.

Expression of mucins in normal salivary glands

In normal salivary glands (fig 1A–F), low expression of all MUC1 isotypes was seen at the surface of the ductal cells but not in the acinar cells (MUC1/DF3; fig 1B). MUC2, MUC4, MUC5AC, and MUC6 were expressed in the epithelium of intralobular ducts, but were not expressed in the acinar cells (fig 1C–F). MUC4 expression in the normal ducts was relatively intense (fig 1D).

Expression of mucins in primary lesions of PA

All types of MUC1 and MUC6 were expressed in the primary lesions of several PAs (tables 2 and 3). In the recurrence group, the numbers of patients classified into the high expression group were as follows: MUC1/CORE, one of nine; MUC1/DF3, four of nine; MUC1/MY.1E12, two of nine; MUC1/glycoprotein, two of nine; MUC1/HMFG-1, one of nine; and MUC6, one of nine (table 2). In the non-recurrence group, the numbers of patients classified into the high expression group were as follows: MUC1/CORE, five of 40; MUC1/DF3, three of 40; MUC1/MY.1E12, six of 40; MUC1/glycoprotein, six of 40; MUC1/HMFG-1, none of 40; and MUC6, four of 40 (table 3).

In contrast, MUC2, MUC4, and MUC5AC showed no expression in most cases, but weakly positive expression in a few cases (tables 2 and 3). No patients were classified into the high expression group for MUC2, MUC4, and MUC5AC

Table 3 Summary of clinicopathological factors and mucin expression in the non-recurrence group (n = 40)

							Mucin expression							
							MUC1							
Age (years)	Sex	Site	Size (cm)*	Margin†	Capsular invasion	Tumour variant‡	CORE	DF3	MY.IE12	GP	HMFG-1	MUC2	MUC4	MUC5AC	MUC6
49.4 (14.7)	6 M, 34 F	17 parotid, 7 SM, 13 palate, 3 other oral mucosa	2.3 (1.2)	26 + 12 −	24 + 16 −	14 A 17 B 9 C	25 − 10 + 5 ++	25 − 12 + 3 ++	18 − 16 + 6 ++	18 − 16 + 6 ++	33 − 7 + 0 ++	38 − 2 + 0 ++	35 − 5 + 0 ++	36 − 4 + 0 ++	26 − 10 + 4 ++

*Maximum diameter; †+, incomplete surgical resection; −, complete surgical resection microscopically; ‡A, stroma rich variant; B, intermediate, C, stroma poor variant.
The positively stained neoplastic cells were graded as follows: −, less than 5% of neoplastic cells stained; +, more than 5% but less than 30% of neoplastic cells stained; ++, more than 30% of neoplastic cells stained.
GP, glycoprotein; SM, submandibular.

in both the recurrence and non-recurrence groups (tables 2 and 3).

There was no significant relation between the expression of each mucin and the following clinicopathological factors: age, size, sex, site, capsular invasion, tumour variant, and positive surgical margin (χ^2 test or Fisher's exact test, data not shown).

In the neoplastic cells, all MUC1 antigens were expressed at the cell membrane and/or cytoplasm of the epithelial/myoepithelial components, and MUC6 was expressed in the cytoplasm. MUC1 expression was occasionally seen in the capsular invasion areas in the patients with high expression of MUC1 (fig 2).

Univariate analysis of clinicopathological features and mucin expression

Using Fisher's exact test, in the primary lesions of the recurrence group, larger tumour size (more than 3.0 cm) (six of nine) and high expression of MUC1/DF3 (four of nine) were significantly more frequent than in the primary lesions of the non-recurrence group (11 of 40 and three of 40; $p = 0.016$ and $p = 0.048$, respectively) (tables 4 and 5).

Table 4 summarises the univariate regression analysis of clinicopathological features. The larger tumour size of primary PAs was a significant risk factor affecting the recurrence of PA ($p = 0.035$; OR = 5.27; 95% CI, 1.12 to 24.85; table 4). Other clinicopathological factors such as capsular invasion and stromal ratio of the tumours showed no significant risk.

Table 5 summarises the univariate regression analysis of mucin expression. High expression of MUC1/DF3 was a significant risk factor for the recurrence of PA ($p = 0.011$; OR = 9.87; 95% CI, 1.69 to 57.61, logistic regression analysis). Expression of the other MUC1 antigens (MUC1/CORE, MUC1/MY.1E12, MUC1/glycoprotein, and MUC1/HMFG-1) was not a significant risk factor for the recurrence of PA. No patients showed high expression of MUC2, MUC4, and MUC5AC in either the recurrence or the non-recurrence groups. MUC6 was not a significant risk factor affecting the recurrence of PA (recurrence group, one of nine; non-recurrence group, four of 40).

Multivariate analysis of factors related to recurrence

For multivariate analysis, we used patient's age, patient's sex, tumour size, tumour variant, and high expression of MUC1/DF3 as the analysed factors because these variables were lower than the cutoff p value that we used ($p < 0.20$) at the univariate level. Table 6 summarises the multivariate analysis for recurrence of PA. High expression of MUC1/DF3 was found to be the only independent significant risk factor to predict the recurrence of PA ($p = 0.021$; OR = 20.92; 95% CI, 1.50 to 291.1).

DISCUSSION

There have been few studies of the clinicopathological risk factors for the recurrence of PA—such as age, stromal ratio, and pseudopodia—that have compared the primary lesions of the recurrence group with those of the non-recurrence group.[2 9–11] This is because it is very difficult to collect the primary tumours of recurrent cases: the initial surgery is often carried out in other hospitals or there is a long interval between the initial surgery and recurrence in most patients with PA. In our present study, we found 23 patients who had recurrent PA lesions in our files, but specimens of the primary lesions were available for only nine patients. Previous reports studying primary lesions in the recurrence group revealed the clinicopathological risk factors for the recurrence of PA,[2 9–11] but they did not perform immunohistochemistry.

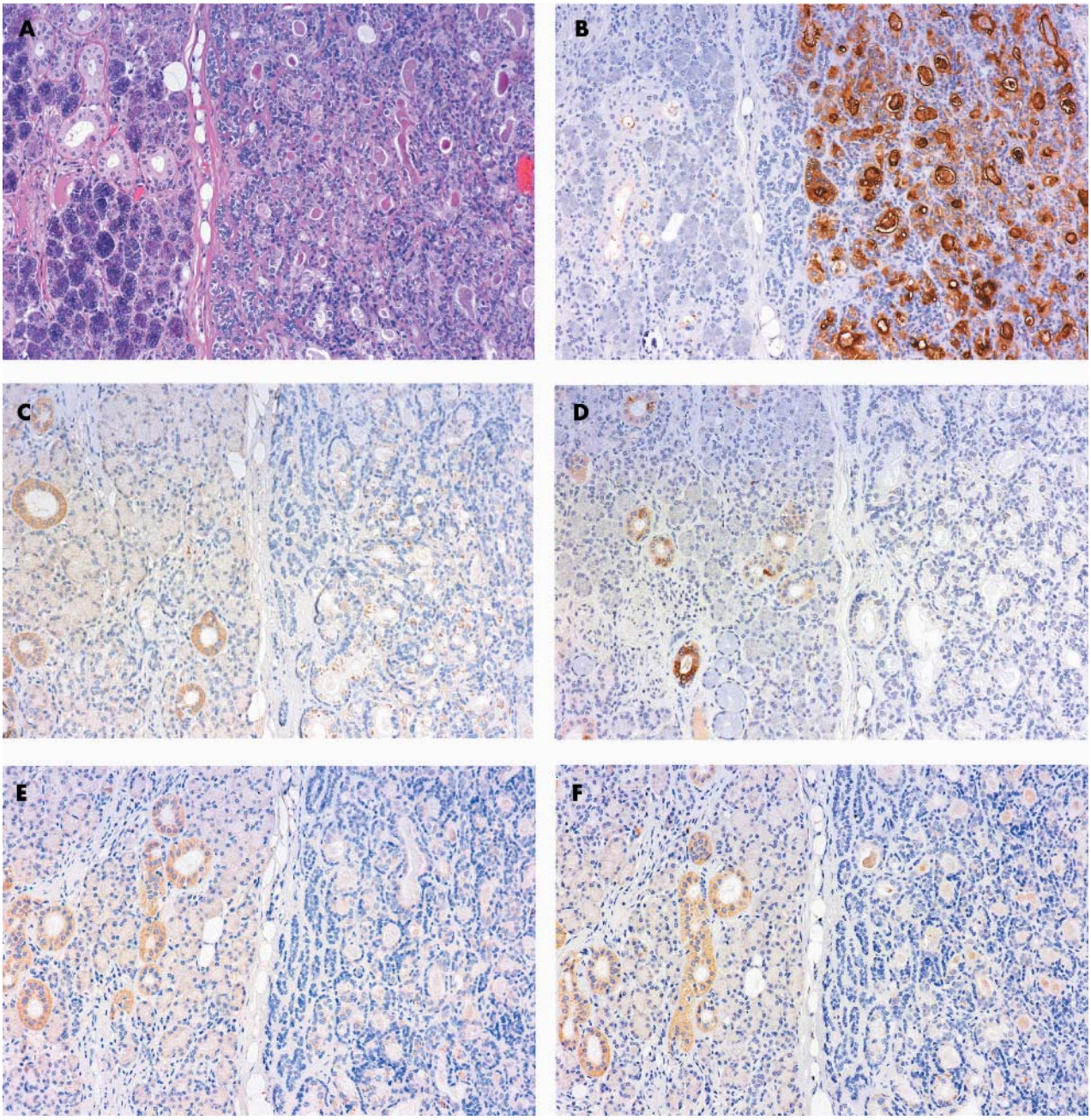

Figure 1 Primary pleomorphic adenoma of the salivary gland in a patient with subsequent recurrence. (A) The tumour cells (right side) were separated from the normal gland (left side) by a thin capsule (haematoxylin and eosin staining). (B) MUC1/DF3 was strongly expressed in the tumour cells. In the normal gland, low expression was seen at the surface of the ductal cells but not in the acinar cells. (C) MUC2, (D) MUC4, (E) MUC5AC, and (F) MUC6 were expressed in the intralobular ducts of the normal salivary gland (left side), but were not expressed in the tumour cells (right side). (D) MUC4 expression in the normal ducts was relatively intense.

In contrast, there have been a few immunohistochemical studies that compared proliferative activity (Ki-67 labelling) or progesterone receptor expression in the secondary recurrent lesions with those in the primary lesions of patients without recurrence.[14 16] However, they did not evaluate factors in the primary lesions of the recurrence group that might predict a recurrence. Our study is the first to use immunohistochemistry on the primary lesions of patients with a subsequent recurrence. Our present study has shown for the first time that high expression of MUC1/DF3, one of the membrane mucin MUC1 antigens, is a significant independent risk factor for the prediction of the recurrence of PA.

In univariate analysis of the clinicopathological factors examined, only tumour size was a significant risk factor for recurrence, and the other risk factors such as younger age[9–11] and stromal rich variant,[11] which have been thought to play an important role in the development of recurrence, did not show a higher incidence of recurrence, as reported by others.[12 13] The relation between incomplete surgical removal (for example, tumour spillage, positive surgical resection margin, and capsule rupture during surgery) with the recurrence of PA is also controversial.[1 2 4 12] Natvig *et al* found that the recurrence rate after capsule rupture was not significantly different from that seen in other patients, and that there was also no difference in the recurrence rate

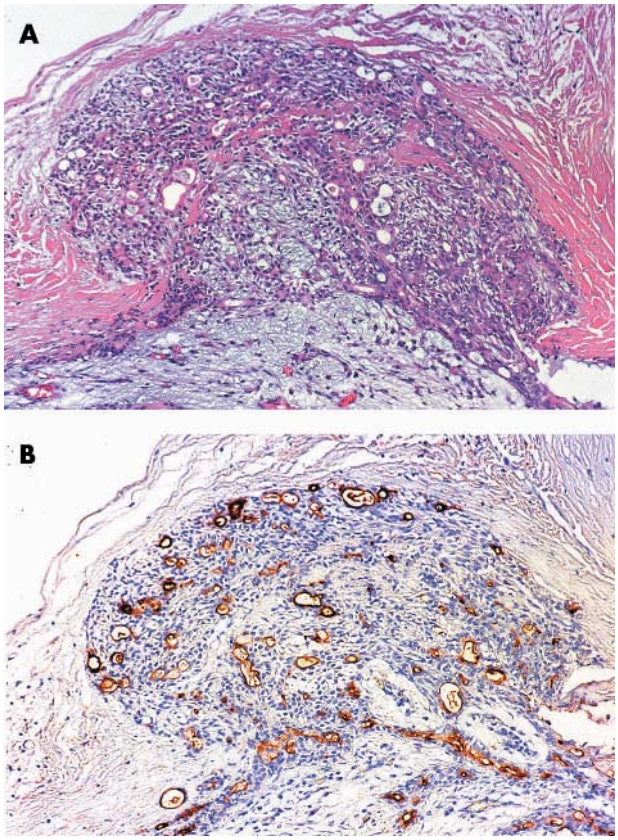

Figure 2 MUC1 expression was occasionally seen in the capsular invasion areas. (A) haematoxylin and eosin staining. (B) MUC/DF3 immunohistochemistry.

between patients with microscopic positive or negative surgical resection margins.[4] Incomplete surgical resection was not a significant risk factor for the recurrence of PA in our study also. Consequently, the mechanism of recurrence of PA is unclear, and we suspect that other intrinsic biological factors might play a more important role in the recurrence of PA.

We found that MUC1/DF3 was overexpressed in several cases of PA, and high expression of this antigen was a significant independent factor for predicting the recurrence of PA; that is, the rate of recurrence after initial surgery was significantly higher in patients with high expression of MUC1/DF3 than in those with low expression.

> ''Our present study has shown for the first time that high expression of MUC1/DF3, one of the membrane mucin MUC1 antigens, is a significant independent risk factor for the prediction of the recurrence of pleomorphic adenoma''

In multivariate analysis, only high expression of MUC1/DF3 was a significant independent risk factor for recurrence. Our previous immunohistochemical studies for mucin expression in various human tumours have shown that MUC1 mucin expression is related to invasive tumour growth and poor patient outcome,[23–28] so that these results agree with our previous findings. MUC1 mucin is a transmembrane glycoprotein with an extracellular domain consisting of a variable number of highly conserved tandem repeats of 20 amino acids, a transmembrane domain, and a cytoplasmic tail of 69 amino acids.[17] Overexpression of MUC1 by cultured cells inhibits their aggregation, possibly because of its large, extended, and rigid structure.[40] The MUC1 expressed in tumours may function as an antiadhesion molecule that inhibits cell–cell adhesion, permitting invasion into surrounding tissues.[40 41] Overexpression of MUC1 on the

Table 4 Univariate analysis of clinicopathological features

Parameters	Recurrence group (%)	Non-recurrence group (%)	Univariate analysis		
			OR	95% CI	p Value
Age (years)					
<35	4 (44)	9 (23)	1		
>35	5 (56)	31 (77)	2.76	0.61 to 12.47	0.19
Size (cm)					
<3.0	3 (33)	29 (73)	1		
>3.0	6 (67)*	11 (27)	5.27	1.12 to 24.85	0.035
Sex					
Male	4 (44)	6 (15)	1		
Female	5 (56)	34 (85)	4.53	0.94 to 21.91	0.060
Site					
Parotid	7 (78)	17 (42)	4.74†	0.87 to 25.72	0.072
Submandibular	1 (11)	7 (17)	–		
Palate	1 (11)	13 (33)	–		
Other oral mucosa	0 (0)	3 (7)	–		
Capsular invasion					
Negative	4 (44)	16 (40)	1		
Positive	5 (56)	24 (60)	1.20	0.28 to 5.16	0.80
Tumour variant					
Stroma rich	6 (67)	14 (35)	3.71‡	0.80 to 17.17	0.096
Intermediate	2 (22)	17 (42)	–		
Stroma poor	1 (11)	9 (23)	–		
Margin					
Negative	4 (44)	26 (65)	1		
Positive	5 (56)	14 (35)	1.49	0.34 to 6.44	0.60

*In Fisher's exact test, the rate of larger tumour size in the primary lesions of the recurrence group was significantly higher than that of the non-recurrence group (p = 0.048); †the referent group was all other sites; ‡the referent group was all other variants.
OR, odds ratio; CI, confidence interval.

Table 5 Univariate analysis of mucin expression

Antibody	Recurrence group (%)	Non-recurrence group (%)	Univariate analysis		
			OR	95% CI	p Value
MUC1/CORE					
LEG	8 (89)	35 (88)	1		
HEG	1 (11)	5 (12)	1.14	0.12 to 11.18	0.91
MUC1/DF3					
LEG	5 (56)	37 (92)	1		
HEG	4 (44)*	3 (8)	9.87	1.69 to 57.61	0.011
MUC1/MY.1E12					
LEG	7 (78)	34 (85)	1		
HEG	2 (22)	6 (15)	0.62	0.10 to 3.72	0.60
MUC1/glycoprotein					
LEG	7 (78)	34 (85)	1		
HEG	2 (22)	6 (15)	0.62	0.10 to 3.72	0.60
MUC1/HMFG-1					
LEG	8 (89)	40 (100)			
HEG	1 (11)	0 (0)	–	–	ND
MUC2					
LEG	9 (100)	40 (100)			
HEG	0 (0)	0 (0)	–	–	ND
MUC4					
LEG	9 (100)	40 (100)			
HEG	0 (0)	0 (0)	–	–	ND
MUC5AC					
LEG	9 (100)	40 (100)			
HEG	0 (0)	0 (0)	–	–	ND
MUC6					
LEG	8 (89)	36 (90)	1		
HEG	1 (11)	4 (10)	0.89	0.09 to 9.06	0.92

*In Fisher's exact test, the rate of high expression of MUC1/DF3 in the primary lesions of the recurrence group was significantly higher than that of the non-recurrence group (p = 0.016).
CI, confidence interval; HEG, the high expression group composed of the ++ cases (more than 30% of the neoplastic cells stained); LEG, the low expression group composed of the − and + cases (less than 30% of neoplastic cells stained); ND, not determined; OR, odds ratio.

membrane of cultured cells may also inhibit interaction between cytotoxic lymphocytes and tumour cells.[42] Cells with high amounts of MUC1 have reduced interaction between integrins and the extracellular matrix.[43] A recent experimental study by Suwa *et al* showed that cultured gastric cancer cells acquire increased motility and invasive abilities when they are transfected with the MUC1 gene.[44] A mechanism for the inhibition of E-cadherin mediated cell–cell adhesion by MUC1 has also been reported.[45] These experimental data may explain the relation between MUC1 mucin expression and the progression of malignant tumours. Our present study showed that MUC1/DF3 expression is also related to tumour recurrence in PA, which is originally a benign tumour.

Table 6 Multivariate analysis of recurrence of PA

Factors	OR	95% CI	p Value
Age (years)			
>35	1		
<35	1.48	0.21 to 10.55	0.70
Size (cm)			
<3.0	1		
>3.0	4.80	0.68 to 33.95	0.12
Sex			
Female	1		
Male	3.58	0.42 to 30.71	0.24
Tumour variant			
Intermediate or stroma poor	1		
Stroma rich	4.07	0.33 to 50.53	0.27
MUC1/DF3			
LEG	1		
HEG	20.92	1.50 to 291.1	0.021

CI, confidence interval; HEG, the high expression group composed of the ++ cases (more than 30% of the neoplastic cells stained); LEG, the low expression group composed of the − and + cases (less than 30% of neoplastic cells stained); OR, odds ratio.

Among the various forms of MUC1 mucins examined, only MUC1/DF3 expression was related to the recurrence of PA, and this difference might be related to the different epitopes recognised by the various antibodies. Tumour cells expressing sialomucin have been shown to be less sensitive to cytolysis by human activated killer lymphocytes,[46–49] and high amounts of cell surface sialomucin may be related to escape from immunological attack.[47 50] MUC1/MY.1E12 is a sialylated MUC1.[36] MUC1/glycoprotein also seems to be a sialylated MUC1 according to the results of the sialidase digestion test in our previous study.[25] A recent study also reported that the MUC1/glycoprotein epitope detected by MAb NCL/MUC-1 (clone Ma695) involves sialic acid.[37] HMFG-1 detects fully glycosylated MUC1 mucin, and its binding is particularly affected by sialic acid.[38] However, the high expression of MUC1/MY.1E12, MUC1/glycoprotein, and HMFG-1 was not related to the recurrence of PA in our present study. The MUC1/DF3 epitope has been thought to involve TRPAPG with a sialylated sugar chain.[35] However, a recent study reported that the MUC1/DF3 epitope does not contain carbohydrate, but core peptide only (APDTRPAP).[34] The MUC1/CORE epitope (detected by clone Ma552) is also a

Take home messages

- MUC1/DF3 expression in pleomorphic adenoma (PA) is a useful marker to predict its recurrence
- Patients with PA who are positive for MUC1/DF3 expression should be followed up carefully
- Prediction of the risk of recurrence by MUC1/DF3 staining in biopsy material may be useful for selecting the surgical procedure

core peptide only (GVTSAPDTRPAP), but high expression of MUC1/CORE was not related to the recurrence of PA. The difference between the MUC1/DF3 epitope and the MUC1/CORE, MUC1/MY.1E12, MUC1/glycoprotein, and MUC1/HMFG-1 epitopes may be responsible for the differences in their association with recurrence of PA. The role of the MUC1/DF3 epitope would be an interesting future area of study.

In conclusion, we report for the first time that the expression of MUC1/DF3 in PA is a useful marker to predict the recurrence of PA. Patients with PA showing positive MUC1/DF3 expression should be followed up carefully. Prediction of the risk of recurrence by MUC1/DF3 staining in biopsy material may be useful for selecting the surgical procedure.

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