

Immunohistochemical study of the expression of MUC5AC and MUC6 in breast carcinomas and adjacent breast tissues

M B Pereira, A J Dias, C A Reis, F C Schmitt

Abstract

Aim—To study the protein expression patterns of MUC5AC and MUC6 in normal and diseased breast tissues and to compare their expression with that of a mucin (MUC1) normally expressed in mammary tissues.

Methods—Formalin fixed, paraffin wax embedded tissue from 69 cases of invasive breast carcinoma and surrounding breast tissue was studied immunohistochemically with monoclonal antibodies against MUC1 (SM3), MUC5AC (CLH2), and MUC6 (CLH6), using the avidin-biotin-peroxidase method.

Results—MUC5AC was detected in five of 68 cases of invasive carcinoma including one of three cases of pure colloid carcinoma. MUC5AC expression in the adjacent normal breast epithelium was present in one of 29 cases and in one of two cases of ductal carcinoma in situ. None of 15 cases of ductal hyperplasia without atypia was positive for MUC5AC. MUC6 was present in 15 of 65 cases of invasive carcinoma, in four of 29 cases of normal adjacent epithelium, two of 15 cases of ductal hyperplasia without atypia, and one of two cases of ductal carcinoma in situ. MUC1 immunoreactivity detected by the SM3 antibody was present in 50 of the 67 cases of invasive carcinoma, but expression was also detected in benign epithelium. All invasive carcinomas expressing MUC5AC were positive for MUC1 and four were positive for MUC6. No significant association was found between the expression of these mucins and tumour size, histological grade, node status, oestrogen receptor status, p53 positivity, or c-ErbB-2 overexpression.

Conclusions—This study documents the expression of two different mucins (MUC5AC and MUC6) not described as being expressed by normal breast tissues in a minority of breast carcinomas, as well as in normal and hyperplastic epithelium. Although the role of mucins in malignant transformation and the progression of breast cancer is not well understood, in some cases, there is probably an upregulation of several genes that encode distinct mucin proteins.

(*J Clin Pathol* 2001;54:210–213)

Keywords: breast carcinoma; mucin expression; MUC5AC; MUC6

Mucins are high molecular weight glycoproteins characterised by a high degree of O-linked carbohydrate chains (50–80% of their mass) and by the presence of a common domain structure that includes one or more regions consisting of tandem repeats, rich in serine and threonine residues, which are potentially O-glycosylated (reviewed in Gendler and Spicer¹ and Kim and colleagues²). Consistent data show that mucins are expressed in a regulated cell and tissue specific manner.^{3–6} The expression of mucin antigens is frequently modified in carcinomas because of alterations in glycosylation.^{1–7} At variance, the mucin core proteins are essentially identical in normal and in neoplastic cells, although upregulation, downregulation, and de novo expression of mucin proteins may occur in cancer cells.^{3–8}

To date, nine mucin genes have been identified.^{2–9} In breast tissues, the epithelial mucin produced by the MUC1 gene is secreted in human milk and it is present in the apical cell membrane of normal epithelium.^{3–9} Several studies have shown that MUC1 is overexpressed in breast carcinomas.^{3–4 10–12} The MUC1 polypeptide is a membrane associated monomeric protein with a short membrane spanning portion followed by tandem repeats containing 25% serine or threonine residues, which are potential glycosylation sites.⁹ In normal tissues, MUC1 is extensively glycosylated, whereas in tumours it is underglycosylated, leading to the accessibility of some antibodies, such as SM3,^{1–7} to epitopes that are not present in normally glycosylated MUC1.

Mucins normally expressed in other tissues have also been studied in breast cancer.^{12–14} Although MUC1^{1–4 12 15 16} and MUC6¹⁴ expression was previously studied by immunohistochemistry, the single study on record regarding the expression of MUC5AC was performed using in situ hybridisation.¹³ In 1997, our group developed a monoclonal antibody CLH2 against a synthetic peptide, based on the MUC5AC tandem repeat, which stained superficial epithelium and cells of the neck glands of the gastric antrum and body, tracheobronchial epithelium, superficial epithelium of the gall bladder, and endocervical epithelium; the single case of normal breast epithelium studied was negative for MUC5AC.⁶

The aim of our present work was to study the pattern of expression of MUC5AC and MUC6 in normal and pathological breast tissues using well characterised monoclonal antibodies^{6 17}

Instituto de Patologia e Imunologia Molecular da Universidade do Porto, Rua Dr Roberto Frias s/n, 4200 Porto, Portugal
M B Pereira
A J Dias
C A Reis
F C Schmitt

Correspondence to:
Dr Schmitt
fernando.schmitt@ipatimup.pt

Accepted for publication
28 June 2000

Table 1 Details of monoclonal antibodies used

Monoclonal antibody	Specificity	Dilution	Ref.
SM3	MUC1	1/10	Burchell <i>et al</i> (1987) ⁹
CLH2	MUC5AC	1/2	Reis <i>et al</i> (1997) ⁶
CLH5	MUC6	1/1	Reis <i>et al</i> (2000) ¹⁷

Monoclonal antibody CLH2 is available from Chemicon Int., Temecula, California, USA.

directed at the tandem repeat portion of these proteins. Furthermore, we have compared the expression of MUC5AC and MUC6 with the cancer associated MUC1 mucin epitope, detected by the monoclonal antibody SM3.

Material and methods

CASE SELECTION

Formalin fixed, paraffin wax embedded tissues from 69 cases of invasive breast carcinoma were obtained from the files of our institute. The patients' ages ranged from 28 to 77 years. All cases were conventionally classified by histological type and the grading was done according to the modified criteria of Bloom and Richardson.¹⁸ In 29 cases, normal adjacent breast tissue was available for study. Areas of ductal hyperplasia without atypia (DHWA) were present in 15 cases. In two cases, ductal carcinoma in situ (DCIS) was also present adjacent to the invasive carcinoma.

IMMUNOHISTOCHEMISTRY

The avidin–biotin–peroxidase complex (ABC) method was used for immunostaining. Briefly, 5 µm sections were cut from paraffin wax blocks, dewaxed, hydrated, and immunostained for MUC1, MUC5AC, MUC6 without antigen retrieval (table 1). For the detection of the oestrogen receptor (1/20; 6F11 antibody; Novocastra, Newcastle, UK), p53 (1/50, DO7 antibody; Dako A/S, Glostrup, Denmark), and c-ErbB-2 (1/200; polyclonal antibody; Dako A/S) we immersed the slides in retrieval solution (Dako A/S) at 100°C for 20 minutes. Endogenous peroxidase activity was quenched by treating the slides with 3% H₂O₂ in methanol for 10 minutes. All primary monoclonal antibodies were applied to the sections and incubated overnight at 4°C. This was followed by incubation with a 1/200 dilution of biotin labelled antimouse secondary antibody (Dako A/S) for 30 minutes and ABC (Dako A/S) also for 30 minutes. The slides were rinsed carefully with Tris buffered saline (TBS) between each step. The colour was developed with diaminobenzidine, and the sections were lightly counterstained with haematoxylin, dehydrated, and mounted.

Negative controls were carried out by omitting the primary antibody. As positive controls, sections from previously studied cases of invasive breast carcinoma known to express MUC1 and normal gastric epithelium known to express MUC5AC and MUC6 were used.

A case was considered to be positive when more than 5% of the cells present in the sections were immunoreactive.^{5,13} A case was considered to have c-ErbB-2 overexpression



Figure 1 MUC5AC immunoreactivity is seen within the cytoplasm of infiltrating ductal carcinoma (avidin–biotin–peroxidase complex method, haematoxylin).

when more than 10% of cells had a complete membranous staining.¹⁹

STATISTICAL ANALYSIS

Chi square and Student's *t* tests were done using Statview 5.0 (SAS Institute Inc, Cary, North Carolina, USA). In all cases, a two tailed *p* < 0.05 was considered significant.

Results

We detected MUC5AC expression in five of 68 cases, including one of three pure colloid carcinomas and four of five invasive ductal carcinomas not otherwise specified (NOS). In one of 29 cases where the normal adjacent breast tissue was present we also detected MUC5AC expression. None of 15 cases of DHWA was positive for MUC5AC. MUC5AC was expressed in one of the two cases of DCIS. The immunostaining pattern was cytoplasmic and granular both in neoplastic and normal cells (fig 1). The proportion of positive tumour cells was variable (5–80%) in the different tumours. The clinicopathological features of the five carcinomas positive for MUC5AC were not significantly different from the negative ones. The five cases expressing MUC5AC were also positive for MUC1 and four were positive for MUC6 (table 2).

MUC1 immunoreactivity (detected by the antibody SM3) was present in 50 of 68 breast cancer cases. MUC 1 expression was also detected in normal adjacent breast epithelium (17 of 29 cases), DHWA (10 of 15 cases), and DCIS (two of two cases). The pattern of staining was heterogeneous and the proportion of positive tumour cells varied greatly between tumours (5–100%). Most invasive carcinomas showed MUC1 expression in the cytoplasm and in the entire membrane.

MUC6 expression was present in 15 of the 65 breast carcinoma cells studied. We also detected MUC6 positivity in four of 29 cases of normal adjacent breast epithelium, two of 15 cases of DHWA, and in one of two cases of DCIS. The immunostaining pattern was cytoplasmic and the intensity was variable.

No significant correlations were found between mucin expression and age, tumour size, histological grading, node status, oestrogen receptor status, or c-ErbB-2 overexpression (table 2).

Table 2 Clinicopathological parameters and MUC1, MUC5AC, and MUC6 expression in breast carcinomas

Clinicopathological features	MUC1		MUC5AC		MUC6	
	+	-	+	-	+	-
No. of cases	50	17	5	63	15	50
Age (years)* (mean (SD))	54.6 (12.4)	53.9 (17.8)	53.4 (8.4)	54.7 (14.4)	52.5 (11.0)	54.6 (14.8)
Tumour size (cm)* (mean (SD))	3.5 (2.2)	4.4 (3.0)	2.9 (0.6)	3.9 (2.5)	3.8 (3.3)	3.9 (2.2)
Histological grade**						
GI	10	3	1	11	1	11
GII	23	7	2	25	9	20
GIII	17	7	2	27	5	19
Node status**						
Positive	29	9	4	35	10	27
Negative	21	8	1	28	5	23
Oestrogen receptor**						
Positive	33	10	2	39	7	33
Negative	17	7	3	24	8	17
p53**						
Positive	22	5	3	27	9	20
Negative	28	12	2	36	6	30
c-erbB2**						
Positive	33	12	1	14	5	10
Negative	10	3	2	42	6	36
Not done	7	2	2	7	4	4

*Not significant (Student's *t* test).**Not significant (χ^2 test).

Discussion

This is the first comprehensive study of MUC5AC immunorexpression in breast cancer. We detected MUC5AC expression in five of 68 cases, which included one pure colloid carcinoma (out of three cases) and four invasive ductal carcinomas (out of 65 cases). In a previous study, O'Connell *et al* showed an altered pattern of mucin expression in mucinous (colloid) breast carcinomas.¹³ Using *in situ* hybridisation, these authors¹³ showed an increase of MUC2 and MUC5AC expression and a decrease of MUC1 expression in colloid breast carcinomas in comparison with non-colloid breast carcinomas. In the study of O'Connell *et al*,¹³ MUC1 and MUC2 expression was studied by immunocytochemistry and *in situ* hybridisation, whereas the expression of MUC5AC was evaluated by *in situ* hybridisation alone. Our results reinforce their observations, showing that MUC5AC is overexpressed in some cases of colloid breast carcinoma. However, at variance with these authors¹³ we found that MUC5AC expression is not restricted to colloid carcinoma cases and was also found in four of the five cases of ductal carcinoma NOS. In a previous report using a monoclonal antibody to MUC5AC, the single case of normal breast evaluated was negative for the MUC5AC mucin.⁶ In our present study, in one case of non-colloid breast carcinoma expressing MUC5AC we also detected MUC5AC expression in the adjacent normal breast epithelium as well as in one area of DCIS. However, we have ignored the expression of MUC5AC in normal breast epithelium adjacent to carcinomas because it might be associated with malignant transformation, reflecting some "field effect" in the pattern of mucin expression, which is not restricted to the colloid histotype.

SM3, an antibody raised against deglycosylated purified milk mucin, was reported to react with almost all breast cancers after fixation in methacarn, but its reactivity can be influenced by formaldehyde.¹¹ Although the sensitivity to fixation procedures may explain

our 17 negative cases of breast carcinoma, the alternative hypothesis that these negative tumours do not have aberrant glycosylation cannot be rule out. SM3 recognises an epitope within the MUC1 tandem repeat that is exposed when the mucin is underglycosylated.^{7,8} As a result of aberrant glycosylation in some carcinomas, the SM3 epitope is selectively expressed in breast, lung, stomach, ovary, and colon neoplasms, whereas it remains masked in the respective normal counterparts.^{7,8} In our series, the expression of the SM3 epitope seen in non-neoplastic tissues surrounding breast carcinomas might reflect the presence of alterations in MUC1 glycosylation, with exposure of the SM3 epitopes in the vicinity of the carcinomas. However, Mommers *et al* described apical positivity for SM3 in normal breast tissues in cases without (pre)invasive carcinomas.¹⁶ This indicates that, assuming that glycosylation was normal in these cases, the SM3 antibody also stains less underglycosylated forms of MUC1.

In our present study, we found the expression of MUC6 in normal and neoplastic breast epithelium. A previous study, by De Bolos *et al*,¹⁴ showed that 57 of the 60 samples of breast carcinoma tested were reactive with anti-MUC6 antibodies. In our series we found MUC6 expression in 15 of the 65 the invasive breast carcinomas. This difference in the proportion of breast carcinomas expressing MUC6 between the two series may be partially explained by differences in antibody reactivity. Moreover, the difference between the studies may stem from the percentage of neoplastic cells used to define positivity for a given case. In our study, a case was considered positive whenever more than 5% of the cells displayed immunostaining.⁵ De Bolos *et al* demonstrated that MUC6 and MUC3 are upregulated by steroids *in vitro* and proposed that the abnormal expression of MUC6 in breast cancers could be partly explained by hormonal changes associated with tumour development.¹⁴ In our series, mucin expression was not significantly correlated with patient's age,

tumour size, histological grade, node status, oestrogen receptor status, p53 status, or c-ErbB-2 overexpression.

In summary, our present study documents the expression of two different mucins (MUC5AC and MUC6), not described as being expressed in normal breast tissues, in a minority of cases of breast carcinoma, as well as the expression of MUC5AC, MUC6, and underglycosylated forms of MUC1 in non-neoplastic breast tissues in the vicinity of breast carcinomas. Although the role of mucins in malignant transformation and the progression of breast cancer are not fully understood, it is possible that in some cases there is an upregulation of several genes that encode distinct mucin proteins. Further studies are needed to clarify the biological role of the expression of "non-mammary mucins", such as MUC5AC and MUC6, in different types of breast lesions, and to evaluate the impact of these results in the prognosis of breast cancer.

We are grateful to Professor L David for helpful discussions and comments on the manuscript. We also would like to thank Dr J Taylor-Papadimitriou and Dr J Burchell for providing the antibody SM3. M B Pereira and A J Dias were supported by Fundação para a Ciência e a Tecnologia through scholarships Praxis XXI—BTI 10610/97 and 10609/97, respectively.

- 1 Gendler SJ, Spicer AP. Epithelial mucin genes. *Annu Rev Physiol* 1995;57:607–34.
- 2 Kim YS, Gum J, Jr, Brockhausen I. Mucin glycoproteins in neoplasia. *Glycoconj J* 1996;13:693–707.
- 3 Ho SB, Niehans GA, Lyftogt C, et al. Heterogeneity of mucin gene expression in normal and neoplastic tissues. *Cancer Res* 1993;53:641–51.
- 4 Audie JP, Janin A, Porchet N, et al. Expression of human mucin genes in respiratory, digestive, and reproductive tracts ascertained by in situ hybridization. *J Histochem Cytochem* 1993;41:1479–85.
- 5 Ho SB, Shekels LL, Toribara NW, et al. Mucin gene expression in normal, preneoplastic, and neoplastic human gastric epithelium. *Cancer Res* 1995;55:2681–90.
- 6 Reis CA, David L, Nielsen PA, et al. Immunohistochemical study of MUC5AC expression in human gastric carcinomas using a novel monoclonal antibody. *Int J Cancer* 1997;74:112–21.
- 7 Girling A, Bartkova J, Burchell J, et al. Core protein epitope of the polymorphic epithelial mucin detected by the monoclonal antibody SM3 is selectively exposed in a range of primary carcinomas. *Int J Cancer* 1989;43:1072–6.
- 8 Reis CA, David L, Seixas M, et al. Expression of fully and under-glycosylated forms of MUC1 in gastric carcinoma. *Int J Cancer* 1998;79:402–10.
- 9 Burchell J, Gendler S, Taylor-Papadimitriou J, et al. Development and characterization of breast cancer reactive monoclonal antibodies directed to the core protein of the human milk mucin. *Cancer Res* 1987;47:5476–82.
- 10 McGuckin MA, Walsh MD, Hohn BG, et al. Prognostic significance of MUC1 epithelial mucin expression in breast cancer. *Hum Pathol* 1995;26:432–9.
- 11 Zotter S, Hageman PC, Lossnitzer A, et al. Tissue and tumour distribution of human polymorphic epithelial mucin. *Cancer Res* 1988;1112:55–101.
- 12 Cao Y, Blohm D, Ghadimi BM, et al. Mucins (MUC1 and MUC3) of gastrointestinal and breast epithelia reveal different and heterogenous tumour-associated aberrations in glycosylation. *J Histochem Cytochem* 1997;45:1547–57.
- 13 O'Connell JT, Shao ZM, Drori E, et al. Altered mucin expression is a field change that accompanies mucinous (colloid) breast carcinoma histogenesis. *Hum Pathol* 1998;29:1517–23.
- 14 De Bolós C, Gumà M, Barranco C, et al. MUC6 expression in breast tissues and cultured cells: abnormal expression in tumours and regulation by steroid hormones. *Int J Cancer* 1998;77:193–9.
- 15 Burchell J, Taylor-Papadimitriou J. Effect of modification of carbohydrate side chains on the reactivity of antibodies with core-protein epitopes of the MUC1 gene product. *Epithelial Cell Biol* 1993;2:155–62.
- 16 Mommers EC, Leonhart AM, von Mensdorff-Pouilly S, et al. Aberrant expression of MUC1 mucin in ductal hyperplasia and ductal carcinoma in situ of the breast. *Int J Cancer* 1999;84:466–9.
- 17 Reis CA, David L, Carvalho F, et al. Immunohistochemical study of the expression of MUC6 mucin and co-expression of other secreted mucins (MUC5AC and MUC2) in human gastric carcinomas. *J Histochem Cytochem* 2000;48:377–88.
- 18 Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I- The value of histological grade in breast cancer: experience from a large study with long-term follow-up. *Histopathology* 1991;19:403–10.
- 19 Press MF, Pike MC, Chazin VR, et al. Her-2/neu expression in node-negative breast cancer: direct tissue quantitation by computerized image analysis and association of overexpression with increased risk of recurrent disease. *Cancer Res* 1993;53:4960–70.

Direct Access to Medline

Medline

Link to Medline from the homepage and get straight into the National Library of Medicine's premier bibliographic database. Medline allows you to search across 9 million records of bibliographic citations and author abstracts from approximately 3,900 current biomedical journals.

www.jclinpath.com