The expression pattern of MUC1 (EMA) is related to tumour characteristics and clinical outcome of invasive ductal breast carcinoma
van der Vegt, Bert; Peterse, J. L.; Patriarca, C.; Hilkens, J.; de Bock, Gertruida H; Wesseling, J.; de Roos, M.A.J.

Published in: Histopathology

DOI: 10.1111/j.1365-2559.2007.02757.x

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date: 2007

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
The expression pattern of MUC1 (EMA) is related to tumour characteristics and clinical outcome of invasive ductal breast carcinoma

B van der Vegt, M A J de Roos, J L Peterse, C Patriarca, J Hilken, G H de Bock & J Wesseling
Department of Pathology and Department of Surgical Oncology, University Medical Centre Groningen, University of Groningen and Department of Pathology, the Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, the Netherlands, Department of Pathology, Azienda Ospedaliera di Melegnano, Milan, Italy, Division of Tumour Biology, the Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam and Department of Epidemiology, University Medical Centre Groningen, University of Groningen, the Netherlands

Date of submission 27 September 2006
Accepted for publication 9 February 2007


The expression pattern of MUC1 (EMA) is related to tumour characteristics and clinical outcome of invasive ductal breast carcinoma

Aims: To clarify MUC1 patterns in invasive ductal breast carcinoma and to relate them to clinicopathological parameters, coexpression of other biological markers and prognosis.

Methods and results: Samples from 243 consecutive patients with primary ductal carcinoma were incorporated into tissue microarrays (TMAs). Slides were stained for MUC1, oestrogen receptor (ER), progesterone receptor (PR), Her2/neu, p53 and cyclin D1. Apical membrane MUC1 expression was associated with smaller tumours (P = 0.001), lower tumour grades (P < 0.001), PR positivity (P = 0.003) and increased overall survival (OS; P = 0.030). Diffuse cytoplasmic MUC1 expression was associated with cyclin D1 positivity (P = 0.009) and increased relapse-free survival (RFS; P = 0.034). Negativity for MUC1 was associated with ER negativity (P = 0.004), PR negativity (P = 0.001) and cyclin D1 negativity (P = 0.009). In stepwise multivariate analysis MUC1 negativity was an independent predictor of both RFS [hazard ratio (HR) 3.5, 95% confidence interval (CI) 1.5, 8.5; P = 0.005] and OS (HR 14.7, 95% CI 4.9, 44.1; P < 0.001).

Conclusions: The expression pattern of MUC1 in invasive ductal breast carcinoma is related to tumour characteristics and clinical outcome. In addition, negative MUC1 expression is an independent risk factor for poor RFS and OS, besides ‘classical’ prognostic indicators.

Keywords: breast carcinoma, ductal, immunohistochemistry, MUC1, prognosis, tissue microarray

Abbreviations: BSA, bovine serum albumin; CI, confidence interval; DCIS, ductal carcinoma in situ; ER, oestrogen receptor; HR, hazard ratio; OS, overall survival; PBS, phosphate-buffered saline; PR, progesterone receptor; RFS, relapse-free survival; TMA, tissue microarray

Introduction

MUC1 (episialin, epithelial membrane antigen, CA15-3 antigen) is a highly O-glycosylated mucin-like transmembrane glycoprotein encoded by a gene on chromosome 1q21. This protein has a very large extracellular domain consisting mainly of 20 amino acid tandem repeats, a transmembrane domain and a cytoplasmic tail.

In most normal glandular epithelial cells, MUC1 is expressed on the apical surface. In vitro and in vivo studies have described cell adhesion inhibition as well as increased metastatic and invasive potential of tumour cells associated with overexpression of MUC1.
MUC1-deficient mice primary breast tumours have a significantly lower growth rate.\textsuperscript{9} Overexpression of an underglycosylated form of MUC1 occurs in nearly all breast carcinomas.\textsuperscript{10–12}

Using numerous different antibodies and scoring methods, many authors have described correlations between MUC1 expression and oestrogen receptor (ER) status, grade of differentiation and prognosis.\textsuperscript{13–15} In contrast with the \textit{in vitro} work, most of these studies have shown a better outcome for patients overexpressing MUC1. Four studies, however, found no relation between MUC1 expression and outcome.\textsuperscript{16–19} These differences may be explained by the complex scoring system used, the different affinity of the applied antibodies for the glycosylated isoforms of MUC1 and the wide range of histopathological phenotypes of breast carcinoma with different clinical and prognostic implications.\textsuperscript{20}

Therefore, we used a monoclonal antibody directed at the protein backbone of MUC1 (mAb 214D4), which is relatively insensitive to the degree and make-up of glycosylation of the molecule\textsuperscript{21} to study five patterns of MUC1 expression in primary ductal carcinomas which were predefined by two of the authors (C.P and J.L.P). To test the potential of this scoring method, it was applied to a set of primary invasive ductal breast carcinomas (not otherwise specified) arranged in a tissue microarray (TMA) and the MUC1 expression patterns were related to clinicopathological parameters, a series of well established biological markers and prognosis. This scoring method has also been applied to a set of ductal carcinomas \textit{in situ} (DCIS).\textsuperscript{22}

\section*{Materials and methods}

\textbf{Patients}

Consecutive patients ($n = 243$) treated for a primary operable invasive ductal carcinoma of the breast (not otherwise specified) at the University Medical Centre Groningen between January 1996 and December 2001 were included in this study. Patient and tumour characteristics and data on follow-up were obtained retrospectively from hospital records and are summarized in Table 1. The median follow-up was 60.5 months (range 0.4–108.2). Follow-up was performed according to the regional follow-up guidelines (http://www.ikcnet.nl/page.php?id=97). During follow-up 12 patients developed a local recurrence after a median follow-up of 26.7 months. Thirty-three patients developed distant metastasis after a median follow-up of 36.7 months. In total, 41 patients presented with a relapse with a median relapse-free survival (RFS) of 27.3 months; 20 patients died due to breast cancer with a median overall survival (OS) of 34.1 months.

\begin{table}[h]
\centering
\begin{tabular}{ll}
\hline
\textbf{Table 1.} Patient and tumour characteristics & \\
\hline
\textbf{Age at diagnosis, median (range)} & 58 (27–89) \\
\hline
\textbf{Menopausal status} & \\
Premenopausal & 75 30.9 \\
Postmenopausal & 168 69.1 \\
\hline
\textbf{Family history} & \\
Positive & 34 14.0 \\
Negative & 157 64.6 \\
Unknown & 52 21.4 \\
\hline
\textbf{Therapy} & \\
Breast-conserving therapy & 145 59.6 \\
Mastectomy & 98 44.9 \\
\hline
\textbf{Axillary nodal status} & \\
Negative & 131 53.9 \\
Positive & 107 44.0 \\
Not assessed & 5 2.1 \\
\hline
\textbf{Pathological tumour size (mm), median (range)} & 20 (2–140) \\
\hline
\textbf{Pathological tumour stage} & \\
T1 & 109 44.9 \\
T2 & 109 44.9 \\
T3 & 18 7.4 \\
Unknown & 7 2.9 \\
\hline
\textbf{Grade of differentiation} & \\
I & 57 23.5 \\
II & 110 45.3 \\
III & 75 30.9 \\
Missing & 1 0.4 \\
\hline
\textbf{Adjuvant chemotherapy} & \\
Yes & 61 25.1 \\
No & 182 74.9 \\
\hline
\textbf{Adjuvant hormonal therapy} & \\
Yes & 87 35.8 \\
No & 156 64.2 \\
\hline
\end{tabular}
\caption{Patient and tumour characteristics}
\end{table}

\textit{n}, Number of cases; T1, tumour diameter < 20 mm; T2, tumour diameter \geq 20 mm but < 50 mm; T3, tumour diameter \geq 50 mm.
Tissue Microarray Construction

From a paraffin block of each tumour, three 0.6-mm core samples of the most representative tumour area were included in a TMA. The technique of TMA production has been described and validated for breast carcinoma by others. In brief, the most representative tumour area was marked on the original haematoxylin and eosin (H&E)-stained section. Using this section for orientation, three 0.6-mm core punches were taken from the selected area in the donor blocks and mounted in a recipient block, using a manual TMA device (Beecher Instruments, Silver Springs, MD, USA).

Immunohistochemistry

Immunohistochemistry for MUC1, ER, progesterone receptor (PR), Her2/neu, cyclin D1 and p53 was performed on these sections. The antibodies and antigen retrieval methods used are summarized in Table 2. The immunohistochemical protocol was as follows: sections were deparaffinized in pure xylene, rehydrated in decreasing concentrations of ethanol and washed in distilled water. Antigen retrieval was performed. Endogenous peroxidase was blocked by incubating in 3% perhydrol for 30 min. The primary antibody diluted in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA) was incubated for 1 h, after which the secondary (1:100 diluted in PBS containing 1% BSA and 1% AB-serum) and tertiary (1:100 diluted in PBS containing 1% BSA and 1% AB-serum) antibodies were incubated for 30 min each. Visualization was performed using the diaminobenzidine tetrahydrochloride/peroxidase reaction. Counterstaining was performed using haematoxylin. Sections were dehydrated using increasing concentrations of alcohol and were mounted.

Evaluation of Immunohistochemistry

Scoring of immunohistochemistry was performed by a resident (B.v.d.V.) and randomly verified by an experienced pathologist (J.W.). ER, PR, p53 and cyclin D1 were graded based on the percentage of tumour cells showing nuclear immunopositivity. ER,

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Supplier</th>
<th>Dilution</th>
<th>Antigen retrieval</th>
<th>Secondary antibody</th>
<th>Supplier</th>
<th>Tertiary antibody</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>MUC1</td>
<td>214D4</td>
<td>Dr J. Hilkens*</td>
<td>1:100</td>
<td>–</td>
<td>RAMPO</td>
<td>Dako</td>
<td>GARPO</td>
<td>Dako</td>
</tr>
<tr>
<td>ER</td>
<td>6F11</td>
<td>Ventana</td>
<td>†</td>
<td>Tris–HCl 0.1 M (pH 9.5) 30 min 98 °C microwave</td>
<td>RAMBIO</td>
<td>Dako</td>
<td>SARBIO</td>
<td>Dako</td>
</tr>
<tr>
<td>PR</td>
<td>1A6</td>
<td>Ventana</td>
<td>†</td>
<td>Tris–HCl 0.1 M (pH 9.5) 30 min 98 °C microwave</td>
<td>RAMBIO</td>
<td>Dako</td>
<td>SARBIO</td>
<td>Dako</td>
</tr>
<tr>
<td>p53</td>
<td>BP-53-12-1</td>
<td>Biogenix</td>
<td>1:800</td>
<td>Tris–HCl 0.1 M (pH 9.5) 30 min 98 °C microwave</td>
<td>RAMBIO</td>
<td>Dako</td>
<td>SARBIO</td>
<td>Dako</td>
</tr>
<tr>
<td>Cyclin D1</td>
<td>SP4</td>
<td>Neomarkers</td>
<td>1:50</td>
<td>Tris–HCl 0.1 M (pH 9.5) 30 min 98 °C microwave</td>
<td>RAMBIO</td>
<td>Dako</td>
<td>SARBIO</td>
<td>Dako</td>
</tr>
<tr>
<td>Her2/neu</td>
<td>CB11</td>
<td>Ventana</td>
<td>†</td>
<td>Tris–HCl 0.1 M (pH 9.5) 30 min 98 °C microwave</td>
<td>RAMBIO</td>
<td>Dako</td>
<td>SARBIO</td>
<td>Dako</td>
</tr>
</tbody>
</table>

ER, Oestrogen receptor; PR, progesterone receptor; RAMPO, rabbit antimouse polyclonal; RAMBIO, rabbit antimouse biotin; GARPO, goat antirabbit polyclonal; SARBIO, swine antirabbit biotin; –, no antigen retrieval necessary.

*Gift from Dr J. Hilkens, Division of Tumour Biology, the Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Plesmanlaan 121, 10066 CX Amsterdam, the Netherlands.
†Prediluted by supplier.
PR and cyclin D1 were considered positive if nuclear staining was present in ≥10% of the cells and p53 was considered positive when there were > 30% of positively stained nuclei. Her2/neu expression was graded as recommended by the manufacturer’s scoring guidelines: 0, no staining at all or membrane staining in <10% of tumour cells; 1+, faint/barely perceptible partial membrane staining in >10% of tumour cells; 2+, weak to moderate complete membrane staining in >10% of tumour cells; 3+, intense complete membrane staining in >10%. Her2/neu was considered positive if the score was 3+. MUC1 was graded according to the five expression patterns depicted in Figure 1. MUC1 expression was

![Figure 1. MUC1 immunoreactivity patterns as classified in this study. A, Entire membrane. B, Apical. C, Focal cytoplasmic. D, Diffuse cytoplasmic. E, 'Inside-out'.](image-url)
considered positive if there was staining in > 10% of tumour cells.

**DATA ANALYSIS**

Data analysis was performed using the SPSS 12.0.1 statistical package (SPSS Inc., Chicago, IL, USA). \( \chi^2 \) tests were used to evaluate the association of MUC1 expression with clinicopathological parameters and biological markers. Because this was an exploratory analysis, a strict significance level was used. Only \( P \)-values (uncorrected) < 0.01 were included. If applicable, Fisher’s exact test was used. In a second analysis, Kaplan–Meier curves were plotted and log-rank scores were calculated. In this analysis, \( P < 0.05 \) was considered to be significant. After this, the six expression patterns were simplified into three subgroups according to expression location: entire membrane, apical membrane and ‘inside-out’ expression were classified as membrane expression; diffuse cytoplasmic and focal cytoplasmic were classified as cytoplasmic expression; tumours negative for MUC1 were classified as negative. These groups and other well-established prognostic indicators were entered into univariate Cox regression analysis to analyse the relationship with RFS and OS. Variables from the univariate analysis with a \( P \)-value of < 0.05 were then entered in a stepwise multivariate Cox regression analysis to investigate the relationship with RFS and OS.

**Results**

**TISSUE MICROARRAY AND IMMUNOHISTOCHEMISTRY**

Of the 243 cases included, the tissue cores of 237 cases were adequately represented in the TMA. Immunohistochemistry could be evaluated in all cases (100%, \( n = 237 \)) for MUC1, p53 and cyclin D1, in 235 cases (99.2%) for Her2/neu, in 232 cases (97.9%) for ER and in 230 cases (97.0%) for PR.

In the assessable cases, MUC1 was expressed in 221 cases (93.2%) showing either a single or a combination of expression patterns. Sixteen cases (6.8%) did not show any expression of MUC1. Entire membrane expression was seen in 48 cases (20.3%). Sixty-four cases (27.0%) showed apical expression. In 21 cases (8.9%), focal cytoplasmic expression was seen. The most common expression was diffuse cytoplasmic (73.0%, \( n = 173 \)). ‘Inside-out’ expression was seen in 23 cases (9.7%), whereas 117 cases (49.4%) showed a single expression pattern. The most common single expression pattern was diffuse cytoplasmic (70.1%, \( n = 82 \)). One hundred cases (42.2%) showed a combination of two patterns and four (1.7%) showed a combination of three expression patterns. The most common combination of expression patterns was apical and diffuse cytoplasmic expression (40.3%, \( n = 42 \)).

**MUC1 EXPRESSION AND CLINICOPATHOLOGICAL PARAMETERS**

The relationship between MUC1 expression pattern and clinicopathological parameters is shown in Table 3. Apical MUC1 expression was associated with smaller tumours (\( P = 0.001 \)) and with lower tumour grades (\( P < 0.001 \)).

**MUC1 EXPRESSION AND BIOLOGICAL Markers**

Table 4 shows the relationship between MUC1 expression and other biological markers.

For apical MUC1 expression a significant association with PR (\( P = 0.003 \)) expression was found. The association with ER was not significant (\( P = 0.049 \)). Diffuse cytoplasmic MUC1 expression showed an association with cyclin D1 (\( P = 0.009 \)). For ‘inside-out’ MUC1 expression a non-significant association with ER was found (\( P = 0.026 \)). Negativity for MUC1 was associated with ER (\( P = 0.004 \)), PR (\( P = 0.001 \)) and cyclin D1 (\( P = 0.009 \)).

**MUC1 EXPRESSION AND CLINICAL OUTCOME**

Kaplan–Meier survival curves showed no significant correlation between MUC1 expression of the entire membrane and OS or RFS. Patients with tumours that had apical MUC1 expression displayed a better OS (\( P = 0.030 \); Figure 2b). No relationship between focal cytoplasmic MUC1 expression and survival was found. Patients with tumours that showed diffuse cytoplasmic MUC1 expression had a better RFS than those with tumours that did not show such expression (\( P = 0.034 \); Figure 2a). For ‘inside-out’ MUC1 expression, no correlation with survival was found. MUC1 negativity was significantly associated with worse RFS (\( P \leq 0.001 \)) and OS (\( P \leq 0.001 \); Figure 2c,d).

**ANALYSIS OF COMBINATIONS OF MUC1 EXPRESSION PATTERNS, CLINICOPATHOLOGICAL PARAMETERS, BIOLOGICAL MARKERS AND CLINICAL OUTCOME**

In order to increase the power of the outcome analysis the expression patterns were simplified into...
<table>
<thead>
<tr>
<th>Table 3. MUC1 expression and clinicopathological parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MUC1 staining</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Menopausal status</td>
</tr>
<tr>
<td>Premenopausal</td>
</tr>
<tr>
<td>Postmenopausal</td>
</tr>
<tr>
<td>(n = 237)</td>
</tr>
<tr>
<td>Family history</td>
</tr>
<tr>
<td>Positive</td>
</tr>
<tr>
<td>Negative</td>
</tr>
<tr>
<td>(n = 186)</td>
</tr>
<tr>
<td>Pathological tumour stage</td>
</tr>
<tr>
<td>T1</td>
</tr>
<tr>
<td>T2</td>
</tr>
<tr>
<td>T3</td>
</tr>
<tr>
<td>(n = 230)</td>
</tr>
<tr>
<td>Tumour grade</td>
</tr>
<tr>
<td>I</td>
</tr>
<tr>
<td>II</td>
</tr>
<tr>
<td>III</td>
</tr>
<tr>
<td>(n = 236)</td>
</tr>
<tr>
<td>Lymph node status</td>
</tr>
<tr>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
</tr>
<tr>
<td>(n = 232)</td>
</tr>
</tbody>
</table>
three patterns on the basis of location of MUC1 expression. Apical membrane expression and ‘inside-out’ expression, which were both associated with a biologically less aggressive profile, were combined with entire membrane expression and classified as membrane expression. Diffuse cytoplasmic expression was combined with focal cytoplasmic expression and classified as cytoplasmic expression. Tumours that did not show MUC1 expression were classified as MUC1–.

In order to evaluate the relation between these three types of MUC1 expression and RFS and OS, the dominant type of MUC1 expression in each tumour was classified as membrane or cytoplasmic expression, or as MUC1–. In the case of multiple expression patterns in one lesion, the dominant type of expression was defined as that displayed by the largest percentage of cells.

The results of univariate Cox regression analysis for RFS are shown in Table 5. Significant relations were found for tumour size [hazard ratio (HR) 2.2, 95% confidence interval (CI) 1.1, 4.5, \( P = 0.03 \) for tumours between 20 and 50 mm; HR 3.8, 95% CI 1.4, 10.2, \( P = 0.009 \) for tumours > 50 mm], tumour grade (HR 2.3, 95% CI 1.2, 4.2, \( P = 0.009 \)), MUC1 expression (HR 3.4, 95% CI 1.5, 8.1, \( P = 0.005 \) for MUC1 negativity), Her2/neu expression (HR 2.8, 95% CI 1.1, 7.1, \( P = 0.03 \)), ER expression (HR 0.5, 95% CI 0.3, 1.0, \( P = 0.05 \)), and PR expression (HR 0.4, 95% CI 0.2, 0.7, \( P < 0.01 \)) and RFS.

Table 6 shows the results from univariate Cox regression analysis for OS. Significant results were found for tumour size (HR 6.6, 95% CI 1.6, 26.4, \( P = 0.008 \) for tumours > 50 mm), tumour grade (HR 3.6, 95% CI 1.5, 8.7, \( P = 0.005 \)), axillary lymph node status (HR 3.0, 95% CI 1.1, 7.8, \( P = 0.03 \)), receipt of adjuvant chemotherapy (HR 3.2, 95% CI 1.1, 8.8, \( P = 0.02 \)), MUC1 expression (HR 6.0, 95% CI 2.2, 16.7, \( P = 0.001 \)), Her2/neu expression (HR 6.3, 95% CI 2.2, 17.5, \( P < 0.001 \)), ER expression (HR 0.3, 95% CI 0.1, 0.8, \( P = 0.02 \)), and PR expression (HR 0.4, 95% CI 0.2, 1.0, \( P = 0.05 \)) and OS.

The results from the multivariate analysis for RFS are shown in Table 7. MUC1 expression (HR 4.6, 95% CI 1.5, 8.5, \( P = 0.005 \) for MUC1 negativity) and PR expression (HR 0.4, 95% CI 0.2, 0.8, \( P = 0.09 \)) were significant independent predictors of RFS.

Table 8 shows the results from multivariate analysis for OS. Axillary lymph node status (HR 4.7, 95% CI 1.7, 13.0, \( P = 0.003 \)), MUC1 expression (HR 14.7, 95% CI 4.9, 44.1, \( P < 0.001 \) for MUC1 negativity) and Her2/neu expression (HR 3.7, 95% CI 1.4, 9.5, \( P = 0.006 \)) were significant independent predictors of OS.
### Table 4. MUC1 expression related to biological markers

<table>
<thead>
<tr>
<th>MUC1 staining Biological markers</th>
<th>Entire membrane MUC1 expression</th>
<th>Apical MUC1 expression</th>
<th>Focal cytoplasmic MUC1 expression</th>
<th>Diffuse cytoplasmic MUC1 expression</th>
<th>&quot;Inside-out&quot; MUC1 expression</th>
<th>All patterns of MUC1 immunoreactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Her2/neu negative</td>
<td>42 (87.5)</td>
<td>162 (86.6)</td>
<td>58 (92.1)</td>
<td>146 (84.9)</td>
<td>20 (95.2)</td>
<td>184 (86.0)</td>
</tr>
<tr>
<td>Her2/neu positive</td>
<td>6 (12.5)</td>
<td>25 (13.4)</td>
<td>5 (7.9)</td>
<td>26 (15.1)</td>
<td>1 (4.8)</td>
<td>30 (14.0)</td>
</tr>
<tr>
<td>(n = 235)</td>
<td>P = 0.874</td>
<td>P = 0.150</td>
<td>P = 0.325*</td>
<td>P = 0.315</td>
<td>P = 1.000*</td>
<td>P = 1.000*</td>
</tr>
<tr>
<td>ER negative</td>
<td>7 (14.9)</td>
<td>46 (24.9)</td>
<td>9 (14.1)</td>
<td>44 (26.2)</td>
<td>3 (14.3)</td>
<td>50 (23.7)</td>
</tr>
<tr>
<td>ER positive</td>
<td>40 (85.1)</td>
<td>139 (75.1)</td>
<td>55 (85.9)</td>
<td>124 (73.8)</td>
<td>18 (85.7)</td>
<td>161 (76.3)</td>
</tr>
<tr>
<td>(n = 232)</td>
<td>P = 0.146</td>
<td>P = 0.049</td>
<td>P = 0.422*</td>
<td>P = 0.316</td>
<td>P = 0.026</td>
<td>P = 0.004*</td>
</tr>
<tr>
<td>PR negative</td>
<td>14 (29.2)</td>
<td>71 (39.0)</td>
<td>13 (21.3)</td>
<td>72 (42.6)</td>
<td>5 (23.8)</td>
<td>80 (38.3)</td>
</tr>
<tr>
<td>PR positive</td>
<td>34 (70.8)</td>
<td>111 (61.0)</td>
<td>48 (78.7)</td>
<td>97 (57.4)</td>
<td>16 (76.2)</td>
<td>129 (61.7)</td>
</tr>
<tr>
<td>(n = 230)</td>
<td>P = 0.209</td>
<td>P = 0.003</td>
<td>P = 0.190</td>
<td>P = 0.018</td>
<td>P = 0.952</td>
<td>P = 0.001</td>
</tr>
<tr>
<td>p53 negative</td>
<td>46 (95.8)</td>
<td>176 (93.1)</td>
<td>62 (96.9)</td>
<td>160 (92.5)</td>
<td>19 (90.5)</td>
<td>203 (94.0)</td>
</tr>
<tr>
<td>p53 positive</td>
<td>2 (4.2)</td>
<td>13 (6.9)</td>
<td>2 (3.1)</td>
<td>13 (7.5)</td>
<td>2 (9.5)</td>
<td>13 (6.0)</td>
</tr>
<tr>
<td>(n = 237)</td>
<td>P = 0.491</td>
<td>P = 0.218</td>
<td>P = 0.629*</td>
<td>P = 0.128*</td>
<td>P = 0.645*</td>
<td>P = 0.070*</td>
</tr>
<tr>
<td>Cyclin D1 negative</td>
<td>12 (25.0)</td>
<td>61 (32.3)</td>
<td>17 (26.6)</td>
<td>56 (32.4)</td>
<td>8 (38.1)</td>
<td>65 (30.1)</td>
</tr>
<tr>
<td>Cyclin D1 positive</td>
<td>36 (75.0)</td>
<td>128 (67.7)</td>
<td>47 (73.4)</td>
<td>117 (67.6)</td>
<td>13 (61.9)</td>
<td>151 (69.9)</td>
</tr>
<tr>
<td>(n = 237)</td>
<td>P = 0.330</td>
<td>P = 0.390</td>
<td>P = 0.448</td>
<td>P = 0.009</td>
<td>P = 0.143</td>
<td>P = 0.009</td>
</tr>
</tbody>
</table>

T1, Tumour diameter < 20 mm; T2, tumour diameter ≥ 20 mm but < 50 mm; T3, tumour diameter ≥ 50 mm.

*Fisher’s exact test.
Discussion

This study investigated the relationship between MUC1 expression patterns in invasive ductal carcinoma of the breast (not otherwise specified), tumour characteristics, expression of a series of well-established tumour markers and clinical outcome. To avoid ambiguous results due to the heterogeneity of breast cancer, we focused on this, by far the most common type of breast cancer.

Expression was found in 93.2% of cases. Apical MUC1 expression was significantly associated with smaller tumours, lower tumour grade, ER positivity and PR positivity. Diffuse cytoplasmic MUC1 expression showed a significant association with PR and cyclin D1 positivity. ‘Inside-out’ MUC1 expression was associated with ER positivity. Negativity for MUC1 was significantly associated with ER negativity, PR negativity and cyclin D1 negativity. Patients with apical
MUC1-expressing tumours and patients with diffuse cytoplasmic MUC1-expressing tumours displayed a significantly increased RFS. Patients with tumours negative for MUC1 showed a significantly decreased RFS and OS on both univariate and multivariate analysis. Before discussing the associations found for the different expression patterns of MUC1 in more detail, it is important to discuss the antibodies that have been used to detect MUC1 in various studies. Almost all anti-MUC1 antibodies used are directed against the O-glycosylated extracellular MUC1 tandem repeat domain. However, the degree and make-up of glycosylation may vary extensively among MUC1+ adenocarcinomas, and the affinity for MUC1 of the vast majority of these antibodies depends on the extent and composition of glycosylation. As a consequence, the variety of anti-MUC1 antibodies used to determine MUC1 expression in breast carcinoma may explain at least some of the discrepancies between various studies, as discussed below.

 Detecting almost all glycosylated MUC1 isoforms is important in studying its significance for tumour progression, relationship to other tumour progression markers and to clinical outcome. Some well-established functions of MUC1, e.g. inhibition of cell–cell and cell–extracellular matrix adhesion, are only to a minor

<table>
<thead>
<tr>
<th>Pathological and biological features</th>
<th>n (%)</th>
<th>HR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour size, mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 20</td>
<td>109 (46.2)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20–50</td>
<td>109 (46.2)</td>
<td>2.2</td>
<td>1.1, 4.5</td>
<td>0.03</td>
</tr>
<tr>
<td>&gt; 50</td>
<td>18 (7.6)</td>
<td>3.8</td>
<td>1.4, 10.2</td>
<td>0.009</td>
</tr>
<tr>
<td>(n = 236)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I and II</td>
<td>167 (69.0)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>75 (31.0)</td>
<td>2.3</td>
<td>1.2, 4.2</td>
<td>0.009</td>
</tr>
<tr>
<td>(n = 242)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Axillary lymph node status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>131 (55.0)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>107 (45.0)</td>
<td>1.6</td>
<td>0.9, 3.0</td>
<td>0.12</td>
</tr>
<tr>
<td>(n = 238)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjuvant radiotherapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>98 (40.3)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>145 (59.7)</td>
<td>1.1</td>
<td>0.6, 2.1</td>
<td>0.76</td>
</tr>
<tr>
<td>(n = 243)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjuvant chemotherapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>123 (50.7)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>120 (49.3)</td>
<td>1.4</td>
<td>0.7, 2.5</td>
<td>0.33</td>
</tr>
<tr>
<td>(n = 243)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUC1 expression</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytoplasmic</td>
<td>144 (60.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Membrane</td>
<td>77 (32.5)</td>
<td>1</td>
<td>0.5, 2.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Negative</td>
<td>16 (6.7)</td>
<td>3.4</td>
<td>1.5, 8.1</td>
<td>0.005</td>
</tr>
<tr>
<td>(n = 237)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Her2/neu</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>225 (94.5)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>13 (5.5)</td>
<td>2.8</td>
<td>1.1, 7.1</td>
<td>0.03</td>
</tr>
<tr>
<td>(n = 238)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>54 (22.9)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>182 (77.1)</td>
<td>0.5</td>
<td>0.3, 1.0</td>
<td>0.05</td>
</tr>
<tr>
<td>(n = 236)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5. (Continued)

<table>
<thead>
<tr>
<th>Pathological and biological features</th>
<th>n (%)</th>
<th>HR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>86 (36.9)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>147 (63.1)</td>
<td>0.4</td>
<td>0.2, 0.7</td>
<td>0.003</td>
</tr>
<tr>
<td>(n = 233)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p53</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>226 (93.8)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>15 (6.2)</td>
<td>1.5</td>
<td>0.5, 4.3</td>
<td>0.5</td>
</tr>
<tr>
<td>(n = 241)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclin D1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>75 (30.9)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>168 (69.1)</td>
<td>0.6</td>
<td>0.3, 1.2</td>
<td>0.2</td>
</tr>
<tr>
<td>(n = 243)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n, Number of cases; HR, hazard ratio; ER, oestrogen receptor; PR, progesterone receptor.

*Because of small numbers, grades I and II were combined.

MUC1-expressing tumours and patients with diffuse cytoplasmic MUC1-expressing tumours displayed a significantly increased RFS. Patients with tumours negative for MUC1 showed a significantly decreased RFS and OS on both univariate and multivariate analysis.

Before discussing the associations found for the different expression patterns of MUC1 in more detail, it is important to discuss the antibodies that have been used to detect MUC1 in various studies. Almost all anti-MUC1 antibodies used are directed against the O-glycosylated extracellular MUC1 tandem repeat domain. However, the degree and make-up of glycosylation may vary extensively among MUC1+ adenocarcinomas, and the affinity for MUC1 of the vast majority of these antibodies depends on the extent and composition of glycosylation. As a consequence, the variety of anti-MUC1 antibodies used to determine MUC1 expression in breast carcinoma may explain at least some of the discrepancies between various studies, as discussed below.

Detecting almost all glycosylated MUC1 isoforms is important in studying its significance for tumour progression, relationship to other tumour progression markers and to clinical outcome. Some well-established functions of MUC1, e.g. inhibition of cell–cell and cell–extracellular matrix adhesion, are only to a minor
For that reason, we used mAb 214D4, a monoclonal antibody which is also directed to the protein backbone of the MUC1 repeat domain, but for which affinity is almost independent of glycosylation status.\textsuperscript{21}

In normal glandular epithelium, MUC1 is expressed at the apical surface.\textsuperscript{5} For that reason, apical expression in breast carcinomas (designated 'pattern B', Figure 1) indicates normal routing of MUC1 molecules and, as a consequence, relatively intact glandular

\begin{table}
\centering
\caption{Univariate analysis of the relation of pathological and biological characteristics with overall survival}
\begin{tabular}{llllll}
\hline
Pathological and biological features & \(n\) (%) & HR & 95\% CI & \(P\)-value \\
\hline
Tumour size, mm & & & & \\
\(< 20\) & 109 (46.2) & 1 & & \\
\(20–50\) & 109 (46.2) & 3.0 & 1.0, 9.2 & 0.06 \\
\(> 50\) & 18 (7.6) & 6.6 & 1.6, 26.4 & 0.008 \\
\((n = 236)\) & & & & \\
Grade* & & & & \\
I and II & 167 (69.0) & 1 & & \\
III & 75 (31.0) & 3.6 & 1.5, 8.7 & 0.005 \\
\((n = 242)\) & & & & \\
Axillary lymph node status & & & & \\
Negative & 131 (55.0) & 1 & & \\
Positive & 107 (45.0) & 3.0 & 1.1, 7.8 & 0.03 \\
\((n = 238)\) & & & & \\
Adjuvant radiotherapy & & & & \\
No & 98 (40.3) & 1 & & \\
Yes & 145 (59.7) & 1.0 & 0.4, 2.4 & 0.95 \\
\((n = 243)\) & & & & \\
Adjuvant chemotherapy & & & & \\
No & 123 (50.7) & 1 & & \\
Yes & 120 (49.3) & 3.2 & 1.1, 8.8 & 0.02 \\
\((n = 243)\) & & & & \\
MUC1 expression & & & & \\
Cytoplasmic & 144 (60.8) & 1 & & \\
Membrane & 77 (32.5) & 0.7 & 0.2, 2.3 & 0.6 \\
Negative & 16 (6.7) & 6.0 & 2.2, 16.7 & 0.001 \\
\((n = 237)\) & & & & \\
Her2/neu & & & & \\
Negative & 225 (94.5) & 1 & & \\
Positive & 13 (5.5) & 6.3 & 2.2, 17.5 & < 0.001 \\
\((n = 238)\) & & & & \\
ER & & & & \\
Negative & 54 (22.9) & 1 & & \\
Positive & 182 (77.1) & 0.3 & 0.1, 0.8 & 0.02 \\
\((n = 236)\) & & & & \\
\hline
\end{tabular}
\end{table}

\begin{table}
\centering
\caption{(Continued) Univariate analysis of the relation of pathological and biological characteristics with overall survival}
\begin{tabular}{llllll}
\hline
Pathological and biological features & \(n\) (%) & HR & 95\% CI & \(P\)-value \\
\hline
PR & & & & \\
Negative & 86 (36.9) & 1 & & \\
Positive & 147 (63.1) & 0.4 & 0.2, 1.0 & 0.05 \\
\((n = 233)\) & & & & \\
p53 & & & & \\
Negative & 226 (93.8) & 1 & & \\
Positive & 15 (6.2) & 2.4 & 0.7, 8.1 & 0.2 \\
\((n = 241)\) & & & & \\
Cyclin D1 & & & & \\
Negative & 75 (30.9) & 1 & & \\
Positive & 168 (69.1) & 0.6 & 0.2, 1.5 & 0.3 \\
\((n = 243)\) & & & & \\
\hline
\end{tabular}
\end{table}

\begin{table}
\centering
\caption{Stepwise multivariate analysis investigating the relation of pathological and biological characteristics with relapse-free survival}
\begin{tabular}{llllll}
\hline
Characteristics & HR & 95\% CI & \(P\)-value \\
\hline
MUC1 expression & & & & \\
Cytoplasmic & 1 & & & \\
Membrane & 1.1 & 0.5, 2.2 & 0.8 \\
Negative & 3.5 & 1.5–8.5 & 0.005 \\
\hline
PR & & & & \\
Negative & 1 & & & \\
Positive & 0.4 & 0.2, 0.8 & 0.09 \\
\hline
\end{tabular}
\end{table}

\(n\), Number of cases; HR, hazard ratio; ER, oestrogen receptor; PR, progesterone receptor.

*Because of small numbers, grades I and II were combined.

extent dependent on MUC1 glycosylation status.\textsuperscript{7,29}
MUC1 in breast cancer

Table 8. Stepwise multivariate analysis investigating the relation of pathological and biological characteristics with overall survival

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axillary lymph node status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>4.7</td>
<td>1.7, 13.0</td>
<td>0.003</td>
</tr>
<tr>
<td>MUC1 expression</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytoplasmic</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Membrane</td>
<td>0.6</td>
<td>0.2, 2.0</td>
<td>0.4</td>
</tr>
<tr>
<td>Negative</td>
<td>14.7</td>
<td>4.9, 44.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Her2/neu</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>3.7</td>
<td>1.4, 9.5</td>
<td>0.006</td>
</tr>
</tbody>
</table>

HR, Hazard ratio; 95% CI, 95% confidence interval.

differentiation. Indeed, in our series, apical MUC1 expression was associated with many indicators of good prognosis and a better OS. The association with lower tumour grade, ER, and PR positivity and the absence of distant metastasis has been described. Some authors have found an increased rate of axillary lymph node negativity and longer RFS for patients with tumours showing apical MUC1 expression. Our data did not confirm these findings. Study size, follow-up, and the patients included might account for this difference, e.g. the study by Hayes et al. included only node-positive patients. In accordance with our series, an increase in OS of patients with tumours showing apical MUC1 expression has been reported elsewhere. One relatively small study found no association between apical expression and clinicopathological variables.

Entire membrane MUC1 expression (designated ‘pattern A’, Figure 1) is more often seen in mucinous carcinomas than in ductal carcinomas of no special type. Although this expression pattern appears to be the effect of misrouting in the MUC1 pathway, no unambiguous results on the role of MUC1 expression on the entire membrane in breast cancer have been described. Whereas Parham et al. have shown that high entire membrane expression of MUC1 is associated with low tumour grade, Rahn et al. have shown the opposite. The former study also found an association with positive lymph node status. In the current study no significant associations between MUC1 entire membrane expression and clinicopathological parameters were found. Entire membrane MUC1 expression did not associate with clinicopathological characteristics and outcome in these series. Two other studies that have looked at the relationship between expression of MUC1 on the entire membrane and outcome have also found no such relation. By combining entire membrane and cytoplasmic MUC1 expression, Rakha et al. were able to show a significant decrease in OS and RFS in this group. We did not perform such a subgroup analysis.

‘Inside-out’ expression (designated ‘pattern E’, Figure 1) for MUC1 was present in a small percentage of the tumours and has been previously described by two of the authors (C.P., J.L.P.). This pattern is specific for invasive micropapillary carcinoma, a subtype of ductal breast carcinoma with a high potential to metastasize to axillary lymph nodes. We found no such relationship, however, nor did we find an association between ‘inside out’ expression and outcome. The small number of cases in these series might account for this.

Diffuse cytoplasmic expression of MUC1 was associated with good prognosis in these series. Previous reports have linked cytoplasmic expression of MUC1 to ER negativity, high Her2/neu expression, decreased RFS and decreased OS. The study by Lundy et al. found that MUC1 cytoplasmic expression was related to ER positivity and lower tumour grade. In this study a positive relationship between MUC1 diffuse cytoplasmic expression and PR and cyclin D1 positivity was found, which might be explained by the common combination of apical and diffuse cytoplasmic expression in these series. Results from the subgroup analysis of combined apical and diffuse cytoplasmic MUC1 expression versus strictly cytoplasmic MUC1 expression show that tumours with diffuse cytoplasmic MUC1 expression have a clinicopathological profile that is usually associated with a worse outcome, but that when this is combined with apical MUC1 expression (i.e. a part of the MUC1 is routed correctly) this negative effect disappears.

Focal cytoplasmic expression of MUC1 has been described in lobular carcinoma. To our knowledge, it has not previously been described in ductal carcinoma. We did not find any relationship between focal cytoplasmic expression (designated ‘pattern C’) and any of the investigated variables.

We observed that tumours negative for MUC1 had a very poor outcome with respect to RFS and OS (Figure 2C,D). In addition, absence of MUC1 expression was associated with absence of ER, PR and cyclin D1. These findings support the observation by Luna-More et al. that tumours negative for MUC1 are high grade, are ER– and PR– and are more frequently associated with positive axillary lymph nodes.
have related low or negative MUC1 expression to higher tumour grade\textsuperscript{13} and poor prognosis.\textsuperscript{38} Inflammatory breast carcinoma patients with MUC1– tumours had a significantly shorter OS.\textsuperscript{39} Remarkably, our MUC1– group of breast carcinomas appears to be a subgroup with poor prognosis that cannot be identified with the common prognostic indicators; for both RFS and OS survival MUC1 negativity was the strongest independent predictor (see Tables 7 and 8).

We also performed a study with MUC1 expression in DCIS (unpublished results). Comparison of those results with the current study reveals some interesting differences. The ‘inside-out’ expression pattern is exclusively seen in invasive ductal carcinomas and not in DCIS. Also in DCIS, no MUC1– tumours were found. As mentioned before, the ‘inside-out’ expression pattern is specific for invasive micropapillary carcinoma. MUC1– tumours are a subgroup of tumours that is non-luminal, non-mucin producing. These tumours are probably fast growing and aggressive and may not have a stage of non-invasive growth that can be easily identified because of early invasion. Loss of MUC1 might play a role in this process of early invasiveness. Remarkably, this seems to be in contrast to in vitro and in vivo data, which show that membranous MUC1 overexpression favours adhesion modulation, invasive potential and metastatic capacity of tumour cells.\textsuperscript{6–9} These effects are very likely due to steric hindrance of adhesion molecules by the high density of large and elongated extracellular MUC1 domains at the cell surface.\textsuperscript{7} Undoubtedly, there are more mechanisms available for acquiring invasive potential, e.g. inactivation of the E-cadherin–β-catenin complex as in invasive lobular breast carcinoma. To investigate a potential relationship between MUC1 and E-cadherin expression, we performed immunohistochemistry for E-cadherin and β-catenin. However, the immunoreactivity of both proteins was too heterogeneous and inconsistent for reliable semiquantitative analysis (data not shown).

In this outcome study, patients were treated in a very heterogeneous manner (radiotherapy, chemotherapy and hormonal therapy) and this may have confounded the results somewhat. However, on univariate analysis radiotherapy is not a predictor for either OS or RFS. On multivariate analysis MUC1 negativity remains an independent predictor of RFS and OS, suggesting an effect independent of adjuvant therapy. Because of the many comparisons presented in Tables 3 and 4, only P-values (uncorrected) < 0.01 are presented. It should be noted that this part of the study was of an exploratory nature, based on the hypothesis that MUC1 expression patterns provide added value in relation to clinicopathological parameters. In addition, the selection of such clinicopathological parameters and biomarkers was based on their established role in the biology of carcinomas and in general breast carcinoma in particular. Therefore, it is hoped that the conclusions of this study will contribute to the optimal determination of the clinical impact of MUC1 expression in invasive ductal breast carcinoma.

Acknowledgement

In fond memory of our esteemed colleague Hans Peterse.

References


