Loss of CD55 Is Associated with Aggressive Breast Tumors

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ABSTRACT

Purpose: CD55 is a complement regulatory protein expressed by cells to protect them from bystander attack by complement. CD55 is overexpressed on some tumor cell lines, and in colorectal carcinomas, it has been shown to be an indicator of poor prognostic.

Experimental Design: A large set of samples (480) from patients with primary operable breast cancer followed for 4–192 months were included in the present study. The prognostic significance of CD55 was then investigated in these tumors using an anti-CD55 monoclonal antibody (RM1) that we raised against a synthetic peptide and a standard immunohistochemistry method.

Results: Ninety-five percent of the breast carcinomas expressed CD55 (RM1) with intensity ranging from weak (51%) to strong (6%). High expression of CD55 was significantly associated with low-grade (grades 1 or 2; \( P = 0.001 \)), lymph node negativity (\( P = 0.031 \)), and good prognosis tumors (Nottingham Prognostic Index < 3.4; \( P < 0.001 \)). Survival analysis showed CD55 overexpression was associated with a more favorable outcome and loss of CD55 being associated with poor survival (\( P = 0.001 \)). Intensity of CD55 expression was significantly correlated (\( P = 0.002 \)) with intensity of CD59 expression (as shown in a previous study) in these series of patients.

Conclusions: In conclusion, we found that loss of both CD55 and CD59 in breast carcinomas is associated with a worse prognosis.

INTRODUCTION

Complement is an enzymatic cascade consisting of 30 serum or cellular components and three activation pathways that results in the release of proinflammatory anaphylatoxins, C3b deposition, and formation of the membrane attack complex, finally leading to cell lysis (1, 2). To avoid autologous complement attack, cells express three complement regulatory proteins, CD59 (protectin), CD55 (decay accelerating factor), and CD46 (membrane cofactor protein; Refs. 1–3).

CD55 or decay accelerating factor is a glycosylphosphatidylinositol-anchored protein composed of 4 NH2-terminal short consensus repeat (SCR) domains plus a threonine/serine-rich region proximal to the cell surface. SCR-2, SCR-3, and SCR-4 are required to bind the C3 convertases, but antibodies binding to SCR3 alone can inhibit CD55 function (4). CD55 binds C3 convertases from both the classical and alternative complement pathways displacing C2b and C3b, preventing C3b deposition, and inhibiting the formation of the membrane attack complex (5). CD55 is expressed on cells throughout the body that are exposed to complement, including erythrocytes, leukocytes, endothelial cells, and epithelial cells (4). Most normal surface epithelia express complement regulatory proteins; however, tumors show variable expression of these proteins. It has been assumed that overexpression of one of the complement regulatory proteins may compensate for the loss of another (6).

Invasive ductal carcinomas of the breast have been described as demonstrating variation in phenotype; some tumors express only one inhibitor, whereas others express various combinations of two or three inhibitors (7, 8). Previous immunohistochemical study performed on the limited number of freshly frozen breast tumors have also shown variable staining of CD55. This reactivity decreased with tumor grade, i.e., more grade 3 breast tumors lack CD55 expression (9). In contrast, in colorectal carcinomas overexpression of CD55 has been shown to be an independent indicator of poor prognosis (10), and there appears to be an inverse association between loss of CD59 and increased expression of CD55 in malignant tumors (11, 12). In our previous immunohistochemical study, we have undertaken on the same series of breast tumors that in these invasive breast carcinomas loss of CD59 correlated with poor survival (13).

To our knowledge, however, there has been no previous study of CD55 expression on the large number of paraffin-embedded breast tumors. Moreover, as none of the anti-CD55 monoclonal antibodies available stained formalin-fixed sections well, this correlation could not be confirmed on this series of breast cancer patients.

In the present study, an anti-CD55 monoclonal antibody (mAb) RM1 was raised against a synthetic peptide, and formalin-fixed, paraffin-embedded sections were examined and determined to show stronger immunoreactivity. The aim of the present study was to assess the prognostic significance of CD55 in the same series of patients with primary operable breast cancer, as previously examined with CD59, using anti-CD55 mAb (RM1) with a standard immunohistochemical method.

MATERIALS AND METHODS

Production of mAb to Synthetic Peptides. An 11-mer peptide (CNTGYKLFGST) was chosen from the SCR domain 3 of CD55. This was coupled to the carriers KLH (keyhole limpet
hemocyanin) and BSA via its NH₂-terminal cystein group using the heterobifunctional cross-linking reagent sulfo-m-Maleimido-
benzoyl-N-hydroxysulfosuccinimide, according to the manufact-
urers instructions (Perbio Science, Cheshire, UK). The peptide-
keyhole limpet hemocyanin conjugates were diazylized against PBS, emulsified with Freund’s adjuvant, and used to immunize BALB/c mice. After three immunizations, test bleeds were taken and serum obtained and tested by ELISA against peptide-BSA conjugates. The serum was compared with prebleeds taken from the same mice prior to immunization.

The spleen was obtained from the mouse with the highest serum titer and fused with the heteromyeloma cell line NS0 as previously described (14, 15) and seeded onto microtiter culture plates. The fusions were then selected on hypoxanthine-aminopterin-in-thymidine medium for 10 days before screening the supernatant against peptide-BSA conjugate. The specific activities of the hy-
bridomas were tested on BSA-conjugated peptide (CNT-
GYKLFGST) and CD55-positive cell lines (791T) using an ELISA method. Positive wells were cloned at 3, 1, and 0.3 cells/well and rescreened for antibody-producing clones. These were then re-
cloned a second time at the same cell dilutions. The isotype of mAb was IgG2b as determined using a Mouse Isotyping Test kit. Su-
permatant was collected from the positive clone RM1 and purified on protein G, eluted with 0.1 M glycine (pH 2.0) and diazylized against PBS before determining the protein concentration.

Western Blotting. The RM1 antibody was tested by West-
ern blot against recombinant CD55 (SCR1-4-Fc. Harris CL 2000). Protein was electrophoresed on 8% SDS-polyacrylamide gel under nonreducing conditions and transferred to nitrocellulose (Amer-
sham Life Sciences) for 1.5 h at 150 mA in transblot apparatus (Bio-Rad). The membrane was blocked for overnight at 4°C in 2% Marvel and 3% BSA (Sigma) and probed with 2 μg/ml anti-CD55 antibodies, RM1, BRIC 216 (IBGRL), and 791T/36. Primary an-
tibody was incubated at room temperature for 1 h in 5% BSA, washed in PBS-Tween (0.1%), and then incubated with peroxidase conju-
gated rabbit antimouse secondary antibody for 1 h at room temperature. The blot was washed three times before developing with enhanced chemiluminescence reagent (Amersham Life Sciences).

Patients and Tissue Collection. The present specimen comprises 480 cases of primary operable invasive breast carcinoma from patients ages ≤ 70 years diagnosed between 1987 and 1992 obtained from the Nottingham Tenovus Primary Breast Carcinoma Series. The tumors are all incised and fixed immediately in neutral formalin and then arrayed in the arrayed sample (sampling error), because of lack of tumor in the arrayed sample (sampling error), damaged tissue, or a total lack of tissue at some array positions (empty spots). Samples from 480 patients, for whom long-term follow-up data were known, were included in this study. Patient characteristics, including age and menopausal status, were also collected and information on local, regional, and distant recurrence, and survival was also retrieved. At the time of diagnosis, their ages ranged from 27 to 70 years (mean, 54.4 years). One hundred fifty-six (34%) patients were premenopausal, and 306 (66%) were postmenopausal from a total of 462 patients with recorded meno-
pausal status.

Of 480 tumors, 106 (22%) were grade 1, 159 (33%) were grade 2, and 215 (45%) were histological grade 3. Axillary lymph nodes had been examined in the 480 patients; 322 (67%) were node negative and 158 (33%) had axillary node metastatic disease. Among 480 patients, 393 (82%) were still alive, 76 (16%) died of breast cancer, and 11 (2%) died from other causes.

Of 480 patients, 411 (85%) showed regional recurrence (in axillary lymph nodes), 407 (84%) showed local recurrence (in the breast), and 397 (82%) developed distant metastases. These pa-
tients have been followed up at 3-month intervals initially, then 6 monthly, then annually for a median period of 99 months, present-
ing a mean survival of 86 months (4–192 months).

The series included 244 (50%) invasive tumors of ductal/no special type (NST), 106 (22%) tubular mixed carcinomas, 1 (0.2%) medullary, 16 (3.3%) atypical medullary, 29 (6%) classical lobular, 2 (0.4%) solid lobular, 4 (0.8%) tubulolobular, 15 (3.1%) lobular mixed, 12 (2.5%) tubular, 7 (1.4%) mucinous, 4 (0.8%) invasive cribriform, 5 (1%) invasive papillary, 15 (3.1%) mixed ductal/NST and lobular, 16 (3.3%) mixed NST and special type, and 4 (2.1%) miscellaneous other invasive tumor types.

Immunohistochemistry. Immunohistochemical detection of CD55 was performed using a routine Streptavidin-biotin peroxi-
dase technique as described previously (13). The mAb RM1 (stock concentration, 1 mg/ml) was used for detection of CD55 with the optimal dilution found to be 1:20. After deparaffinization in xylene and rehydration through graded alcohol, slides were immersed in methanol/hydrogen peroxide for 10 min to block endogenous per-
oxidase activity. Sections were immersed in 10 mM sodium citrate buffer (pH 6.0) and heated for 10 min at high power followed by 10 min at low power in a microwave oven at 800 W to retrieve antigenicity. The slides were then incubated with 100 μl of normal swine serum for 10 min to block the nonspecific antibody binding sites. The test sections were then treated with 100 μl of RM1 mAb (1:20 dilution) for 60 min at room temperature. Sections were then incubated in biotinylated goat antimouse secondary antibody (Dako, Ely, Cambridge, UK) diluted 1:100 in normal swine serum for 30 min followed by Streptavidin-biotin/horseradish peroxidase complexes (Dako) for 60 min at room temperature and addition of 3,3'-diaminobenzidine (Dako Liquid DAB plus) to achieve visu-
alization of the antigen. Slides were counterstained with hematox-
ylin (Dako Ltd.), dehydrated in alcohol, cleared in xylene (Genta Medica, York, UK), and mounted with distyrene, plasticiser, and xylene (BDH, Poole, UK). Normal breast tissue adjacent to tumor was used as positive control and omission of the primary antibody and replacing with normal swine serum was used as negative control.

A pilot study of 20 cases was examined on whole tissue sections to assess heterogeneity of the immunoreactivity with the antibody. A comparison of tissue array and original tissues showed similarity in intensity and the area of positivity of CD55 immunoreactivity.

**Scoring of Carcinomas.** The intensity of the staining was estimated on a scale as 0 (absent), 1 (weak), 2 (moderate), and 3 (strong). The percentage of tumor cells showing positivity was assessed semiquantitatively as 1 (<25%), 2 (25–50%), 3 (51–75%), or 4 (>75%). The histochemical score of immunoreactivity was obtained by multiplying the intensity and area values (24). The histochemical score was subgrouped into three equal ranges for analysis, and a score of <100 was considered weak, 100–200 as moderate, and 201–300 as strong.

Immunostaining of CD55 was evaluated using semiquantitative systems in the resection specimens by one author (Z. Madjd) after a series were examined on a double-headed microscope blinded to patients outcome and other clinical finding. Additional cases were also assessed by two observers (Z. Madjd, S. E. Pinder) to confirm agreement at the end of the study.

**Statistical Analysis.** The correlation between CD55 expression levels and other prognostic parameters was statistically analyzed by means of the Pearson R test and Pearson χ² tests. Survival rates were examined by the Kaplan-Meier method for analysis of censored data. The statistical significance of differences between the survival rates of groups with different CD55 expression was analyzed using the log-rank test. The independent prognostic significance of parameters was assessed in multivariate analysis by means of a Cox regression analysis. Ps < 0.05 were assumed statistically significant.

**RESULTS**

Previous small studies on frozen breast sections had suggested that expression of CD55 was variable and may correlate with tumors prognosis. In Nottingham, we have a series of well-defined breast cancer patients with 15 years follow-up. However, the tumor specimens are all formalin fixed, and most

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**Fig. 1** A, ELISA showing titration of RM1 antiserum against purified CD55. ELISA plates were coated with 5 μg/ml purified CD55 overnight at 4°C. Protein was detected with mouse antiserum and detected with rabbit antimouse horseradish peroxidase conjugate and absorbance measured at 405 nm. B, Western Blot analysis of decay accelerating factor fusion protein (SCR 1-4Fc) with RM1 monoclonal antibody. Fifty μg/ml SCR 1-4Fc were loaded onto 8% SDS-PAGE gels under nonreducing conditions and transferred to a nitrocellulose matrix. Blots were detected with anti-decay accelerating factor antibodies at a concentration of 2 μg/ml, horseradish peroxidase conjugated antimouse, and developed with ECL reagents. C, immunohistochemical staining of serial breast sections stained with RM1 with and without preincubation with RM1 peptide (original magnification, ×400). Specific peptide was used (at 5, 2, 1, and 0.5 μg/ml) to block CD55 to determine any nonspecific binding, and no staining was observed, indicating that the monoclonal antibody anti-SCR3 was specific for the regions to which it was produced.
CD55 antibodies do not recognize denature CD55. We therefore raised a mAb against CD55 to stain a panel of 480 breast arrays and correlate expression with prognostic factors.

**Generation of mAb RM1.** Kyte-Doolittle analysis of the third SCR domain of CD55 revealed that the 11-mer peptide with the sequence (CNTGYKLFGST) was the most hydrophilic region. This region is therefore likely to be surface exposed and form a site for antibody intervention. This 11-mer peptide was linked to keyhole limpet hemocyanin and serum was screened for anti-CD55 antibodies by ELISA (Fig. 1A). After three immunizations, titer levels were consistently high at >1/5000. Splenocytes were fused to NS0 and hybridomas screened on CD55. Upon generation of stable expressing clones, RM1 was chosen on its antibody yield. Antibody was purified and tested on nonreducing Western blot against recombinant CD55-Fc (Ref. 25; Fig. 1B).

This was compared with other well-characterized anti-CD55 antibodies, BRIC 216 and 791T/36. All three antibodies were able to detect CD55 by Western blot with the greatest intensity of binding being shown by BRIC 216. Probing the blot with secondary antibody alone (Fig. 1B, Lane C) did not reveal any bands indicating specificity of the antibody.

**Immunohistochemical Expression of CD55 in Breast Tumor Cells.** To test the specificity of the antibody on histological sections, serial sections were cut and incubated with the antibody in the presence/absence of the peptide it was raised against. The results show that the mAb anti-SCR3 was specific for the regions to which it was produced because the staining pattern was completely abolished when the antibody was incubated in the presence of the peptide (Fig. 1C).

Immunostaining was performed and showed that the antibody recognized denatured CD55 and that epitopes were not destroyed by the fixation procedure. Compared with commercial mAb to CD55 (clone 67), which was used as a control, the reaction was much stronger (Fig. 2).

CD55 expression was found in 455 of the 480 invasive breast carcinomas investigated (95%). Weak and moderate CD55 staining was observed in 51% \((n = 246)\) and 37% \((n = 180)\) of tumors, whereas only 6% \((n = 29)\) of breast tumors exhibited strong immunoreactivity (Table 1, Fig. 3). A variable percentage of positive cells staining with CD55 was observed in this series of breast carcinomas; 45% \((n = 216)\) of cases showed extensive expression of CD55 (>75% positive cells), whereas 20% \((n = 98)\) showed CD55 immunoreactivity in <25% of tumor cells (Table 2).

**Correlation of CD55 Expression with Breast Cancer Prognostic Factors.** The relationship between CD55 expression and different prognostic parameters (histological grade, tumor type, vascular invasion, lymph node stage, tumor size, and NPI), patient characteristics (age and menopausal status), and outcome (overall survival and distant metastases, local and regional recurrence) was investigated in 480 breast carcinomas (Table 3). A significant correlation \((P = 0.001)\) was observed between the intensity of CD55 expression and histological grade of invasive tumor, i.e., a strong intensity of CD55 was more often found in grade 1 (G1) tumors (8%) compared with grade 3 (G3) lesions (4%; Table 4).

Moreover, CD55 intensity was significantly \((P = 0.031)\) correlated with lymph node metastatic disease; strong immunoreactivity with CD55 was more often found in lymph node-negative breast tumors (8%) compared with lymph node-positive tumors (2%; Table 4). A significant correlation \((P = 0.001)\) was also observed between CD55 expression and smaller breast tumor (tumor size < 20 mm). Furthermore, a significant correlation \((P < 0.001)\) was evident between CD55 expression and NPI; high expression of CD55 was more often found in tumors from patients in the good and moderate prognosis groups compared with poor prognosis patients (Table 4).

Intensity of CD55 expression was correlated with histological tumor type group \((P = 0.017)\); strong intensity of CD55 was more

<table>
<thead>
<tr>
<th>Table 1 Intensity of CD55 expression</th>
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</thead>
<tbody>
<tr>
<td>Immunohistochemical score</td>
</tr>
<tr>
<td>None</td>
</tr>
<tr>
<td>Weak (+)</td>
</tr>
<tr>
<td>Moderate (+ +)</td>
</tr>
<tr>
<td>Strong (± +)</td>
</tr>
<tr>
<td>Total</td>
</tr>
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</table>
often seen in excellent prognosis type tumors (tubulolobular, tubular, mucinous, and invasive cribriform) compared with poor prognosis types (ductal/NST, solid lobular, lobular mixed, mixed NST, and lobular; Table 4).

In contrast, no correlation was demonstrated between intensity of CD55 expression and the absence or presence of vascular invasion, development of distant metastasis, local and regional recurrence, menopausal status, or patient age at time of diagnosis.

A significant correlation \( (P = 0.002) \) was found between the intensity of CD55 and CD59 expression in these series of patients. CD55 and CD59 showed essentially similar intensities, indicating that loss of both CD55 and CD59 was associated with a more aggressive breast tumor phenotype (13). No correlation \( (P = 0.777) \) was, however, found between the percentage of cells showing immunoreactivity for CD55 and CD59.

**Survival Analysis.** Correlation between the intensity of CD55 expression, grouped as high expression (strong and moderate intensity) and low expression (no/weak intensity) and overall survival for the 480 breast cancer patients, with mean 7 years follow-up, was assessed. Analysis based on the log-rank test revealed a significant correlation \( (P = 0.0016) \) between the intensity of CD55 expression and survival. As can be seen in the corresponding Kaplan-Meier survival plot, (Fig. 4), patients with invasive breast carcinomas showing a high intensity of expression of CD55 had a more favorable outcome than those with tumors with low CD55 expression. In contrast, no association was found between the percentage of cells expressing CD55 and patient survival \( (P = 0.449) \).

![Fig. 3 Expression of CD55 (RM1) in breast carcinomas. A–D, original magnification, × 400. A, absent CD55 expression in breast carcinoma. B, weak expression of CD55 in breast carcinoma. C, moderate staining of CD55 in breast carcinoma. D, strong expression of CD55 in breast carcinoma.](image)

**Table 2** Percentage of cells showing immunoreactivity of CD55

<table>
<thead>
<tr>
<th>Percentage of CD55-positive cells classified in four groups</th>
<th>% of tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;25% positive</td>
<td>20 (n = 98)</td>
</tr>
<tr>
<td>25–50% positive</td>
<td>15 (n = 74)</td>
</tr>
<tr>
<td>51–75% cells</td>
<td>19 (n = 92)</td>
</tr>
<tr>
<td>&gt;75% cells</td>
<td>45 (n = 216)</td>
</tr>
<tr>
<td>Total</td>
<td>480</td>
</tr>
</tbody>
</table>

**Table 3** Correlation of CD55 expression in invasive breast carcinoma with clinicopathological parameters (Pearson’s R test)

<table>
<thead>
<tr>
<th>Prognostic factors</th>
<th>Cutoff points</th>
<th>Percentage of cells stained (P)</th>
<th>Intensity of staining (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histological grade</td>
<td>1, 2, or 3</td>
<td>0.003</td>
<td>0.001</td>
</tr>
<tr>
<td>Lymph node (LN)</td>
<td>LN−/LN+</td>
<td>0.844</td>
<td>0.031</td>
</tr>
<tr>
<td>Tumor size (mm)</td>
<td>&lt;10, 11–20, 21–30, 31–40, 41–50</td>
<td>0.631</td>
<td>0.01</td>
</tr>
<tr>
<td>NPI*</td>
<td>Good, moderate, or poor</td>
<td>0.276</td>
<td>0.000</td>
</tr>
<tr>
<td>Distant metastases</td>
<td>Absent or present</td>
<td>0.291</td>
<td>0.109</td>
</tr>
<tr>
<td>Local recurrence</td>
<td>Absent or present</td>
<td>0.051</td>
<td>0.830</td>
</tr>
<tr>
<td>Regional recurrence</td>
<td>Absent or present</td>
<td>0.162</td>
<td>0.883</td>
</tr>
<tr>
<td>Vascular invasion</td>
<td>None or definite</td>
<td>0.231</td>
<td>0.575</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>&lt;40, 41–50, 51–60, &gt;60</td>
<td>0.301</td>
<td>0.676</td>
</tr>
<tr>
<td>Menopausal status</td>
<td>Pre- or postmenopausal</td>
<td>0.917</td>
<td>0.547</td>
</tr>
<tr>
<td>Tumor type(^b)</td>
<td>1, 2, 3, or 4</td>
<td>0.035</td>
<td>0.017</td>
</tr>
</tbody>
</table>

\(^a\) The Nottingham Prognostic Index (NPI) is an integrated prognostic index used to predict patient survival for women with invasive breast cancer based on tumor size, lymph node stage, and tumor grade. It is often clinically classified in three groups: good (≤3.4); moderate (3.41–5.4); and poor prognosis (>5.4).

\(^b\) Tumor type classified in four prognostic type groups: 1, excellent prognosis type (>80%, 10-year survival) includes tubulolobular, tubular, mucinous, and invasive cribriform; 2, good types (60–80%, 10-year survival) includes tubular mixed, alveolar lobular, mixed ductal, and special type; 3, moderate prognosis types (50–60%, 10-year survival) includes medullary, atypical medullary, and classical lobular, invasive papillary, and 4, poor prognosis types (<50%, 10-year survival) includes ductal, solid lobular, lobular mixed, mixed ductal, and lobular.

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**Fig. 3** Expression of CD55 (RM1) in breast carcinomas. A–D, original magnification, × 400. A, absent CD55 expression in breast carcinoma. B, weak expression of CD55 in breast carcinoma. C, moderate staining of CD55 in breast carcinoma. D, strong expression of CD55 in breast carcinoma.
Loss of CD55 and Aggressive Breast Tumors

also correlated with poor prognosis. The present study adds additional insight in the expression of complement regulatory protein CD55 in the same series of nomas showing variable expression of complement regulatory protein CD59 (protectin) and loss of CD59 have a poor survival. Recently, we have reported that patients with breast carcinoma showing variable expression of complement regulatory protein CD59 (protectin) and loss of CD59 have a poor survival. A marked variation in intensity of CD55 reactivity was observed, ranging from weak (51%) to strong (6%). This variable expression of CD55 by breast tumors has been noted in a number of immunohistochemical studies. For example, Hofman et al. (9) showed a complete loss of CD55 expression and strong reactivity to CD46 in malignant tumors.

Moreover, we found a significant correlation between intensity of CD55 expression and histological grade, NPI, lymph node stage, tumor type, and with overall patient survival. Loss of CD55 was more often found in high-grade and poor prognosis group (by NPI) breast carcinomas, and it was more frequently seen in lymph node-positive patients. Overall, patients with high levels of CD55 expression, when compared with patients with low expression, had a significantly better survival (P = 0.001).

In this series of patients, the intensity of CD59 expression was also correlated with histological grade and NPI, i.e., carcinomas exhibiting a high grade and identified as being in the poor prognostic group lacked CD59. Our data indicated that loss of both CD55 and CD59 was more often observed in malignant tumors using mAb RM1 and shows that loss of CD55 is also correlated with poor prognosis.

We observed a widely varying percentage and intensity of expression of CD55 in this series of breast tumors. Forty-five percent of breast carcinomas showed a high expression of CD55 (>75% positive cells), whereas 20% of cases expressed CD55 in <25% of tumor cells. A marked variation in intensity of CD55 reactivity was observed, ranging from weak (51%) to strong (6%). This variable expression of CD55 by breast tumors has been noted in a number of immunohistochemical studies. For example, Hofman et al. (9) showed a complete loss of CD55 expression and strong reactivity to CD46 in malignant tumors.

Moreover, we found a significant correlation between intensity of CD55 expression and histological grade, NPI, lymph node stage, tumor type, and with overall patient survival. Loss of CD55 was more often found in high-grade and poor prognosis group (by NPI) breast carcinomas, and it was more frequently seen in lymph node-positive patients. Overall, patients with high levels of CD55 expression, when compared with patients with low expression, had a significantly better survival (P = 0.001).

In this series of patients, the intensity of CD59 expression was also correlated with histological grade and NPI, i.e., carcinomas exhibiting a high grade and identified as being in the poor prognostic group lacked CD59. Our data indicated that loss of both CD55 and CD59 was more often observed in malignant tumors. It is uncertain if loss of expression of CD55 is because of selective pressure from immune surveillance or is a consequence of gene mutation leading to alter transcriptional control within cancer cells.

CD55 is expressed by cells to protect them from bystander attack by complement. The absence of CD55 and CD59 may leave tumor cells vulnerable to complement-mediated lysis. However, it has been observed that loss of one complement inhibitory protein may be compensated by an increased expression of one of the others (6). CD55 and CD46 (membrane cofactor protein) cooper-

<table>
<thead>
<tr>
<th>Intensity of CD55 expression</th>
<th>No</th>
<th>Weak</th>
<th>Moderate</th>
<th>Strong</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Grading</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>(n = 107)</td>
<td>4 (4%)</td>
<td>47 (44%)</td>
<td>47 (44%)</td>
<td>9 (8%)</td>
</tr>
<tr>
<td>G2</td>
<td>(n = 158)</td>
<td>8 (5%)</td>
<td>66 (42%)</td>
<td>73 (46%)</td>
<td>11 (7%)</td>
</tr>
<tr>
<td>G3</td>
<td>(n = 215)</td>
<td>13 (6%)</td>
<td>130 (61%)</td>
<td>63 (29%)</td>
<td>9 (4%)</td>
</tr>
<tr>
<td><strong>Lymph node stage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>(n = 319)</td>
<td>18 (6%)</td>
<td>150 (47%)</td>
<td>126 (39%)</td>
<td>25 (8%)</td>
</tr>
<tr>
<td>Positive</td>
<td>(n = 157)</td>
<td>6 (4%)</td>
<td>92 (59%)</td>
<td>55 (35%)</td>
<td>4 (2%)</td>
</tr>
<tr>
<td><strong>NPI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>(n = 172)</td>
<td>9 (5%)</td>
<td>70 (41%)</td>
<td>78 (45%)</td>
<td>15 (9%)</td>
</tr>
<tr>
<td>Moderate</td>
<td>(n = 240)</td>
<td>12 (5%)</td>
<td>126 (53%)</td>
<td>89 (37%)</td>
<td>13 (5%)</td>
</tr>
<tr>
<td>Poor</td>
<td>(n = 54)</td>
<td>3 (6%)</td>
<td>40 (74%)</td>
<td>10 (18%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td><strong>Tumor type</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excellent</td>
<td>(n = 22)</td>
<td>1 (4%)</td>
<td>7 (32%)</td>
<td>10 (46%)</td>
<td>4 (18%)</td>
</tr>
<tr>
<td>Good</td>
<td>(n = 109)</td>
<td>2 (2%)</td>
<td>58 (53%)</td>
<td>47 (43%)</td>
<td>2 (2%)</td>
</tr>
<tr>
<td>Moderate</td>
<td>(n = 38)</td>
<td>1 (3%)</td>
<td>21 (55%)</td>
<td>14 (37%)</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Poor</td>
<td>(n = 256)</td>
<td>20 (8%)</td>
<td>139 (54%)</td>
<td>82 (32%)</td>
<td>15 (6%)</td>
</tr>
</tbody>
</table>

Table 4 Correlation of intensity of CD55 expression with histological grade, lymph node stage, Nottingham Prognostic Index (NPI), and tumor type

To investigate whether CD55 expression had independent prognostic significance, Cox multivariate regression analysis, including the parameters of histological grade and lymph node stage with CD55 expression, was performed. These analyses demonstrated that while lymph node stage (P < 0.001) and grade (P < 0.001) were of independent prognostic significance for survival of patients with invasive breast carcinoma, CD55 expression (P = 0.41) was not an independent prognostic parameter (Table 5).

DISCUSSION

Recently, we have reported that patients with breast carcinomas showing variable expression of complement regulatory protein CD59 (protectin) and loss of CD59 have a poor survival. The present study adds additional insight in the expression of complement regulatory protein CD55 in the same series of breast tumors using mAb RM1 and shows that loss of CD55 is also correlated with poor prognosis.

We observed a widely varying percentage and intensity of expression of CD55 in this series of breast tumors. Forty-five percent of breast carcinomas showed a high expression of CD55 (>75% positive cells), whereas 20% of cases expressed CD55 in <25% of tumor cells. A marked variation in intensity of CD55 reactivity was observed, ranging from weak (51%) to strong (6%). This variable expression of CD55 by breast tumors has been noted in a number of immunohistochemical studies. For example, Hofman et al. (9) showed a complete loss of CD55 expression and strong reactivity to CD46 in malignant tumors.

Moreover, we found a significant correlation between intensity of CD55 expression and histological grade, NPI, lymph node stage, tumor type, and with overall patient survival. Loss of CD55 was more often found in high-grade and poor prognosis group (by NPI) breast carcinomas, and it was more frequently seen in lymph node-positive patients. Overall, patients with high levels of CD55 expression, when compared with patients with low expression, had a significantly better survival (P = 0.001).

In this series of patients, the intensity of CD59 expression was also correlated with histological grade and NPI, i.e., carcinomas exhibiting a high grade and identified as being in the poor prognostic group lacked CD59. Our data indicated that loss of both CD55 and CD59 was more often observed in malignant tumors. It is uncertain if loss of expression of CD55 is because of selective pressure from immune surveillance or is a consequence of gene mutation leading to alter transcriptional control within cancer cells.

CD55 is expressed by cells to protect them from bystander attack by complement. The absence of CD55 and CD59 may leave tumor cells vulnerable to complement-mediated lysis. However, it has been observed that loss of one complement inhibitory protein may be compensated by an increased expression of one of the others (6). CD55 and CD46 (membrane cofactor protein) cooper-

<table>
<thead>
<tr>
<th>Clinical factors</th>
<th>Hazard ratio (95% confidence interval)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor size</td>
<td>1.11 (0.84–1.47)</td>
<td>0.46</td>
</tr>
<tr>
<td>Stage</td>
<td>8.17 (2.5–26.47)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD55 intensity</td>
<td>6.76 (3.74–12.13)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD55 intensity</td>
<td>1.24 (0.74–2.06)</td>
<td>0.41</td>
</tr>
</tbody>
</table>
atively protect host cells from C3 targeting in complement cascade. It is speculated that because CD46 alone sufficiently inhibits the complement cascade at an early stage in some cell lines (26), the expression of other complement inhibitors that act during later steps might be unnecessary.

In colorectal cancer, it has been shown that CD55 overexpression is an indicator of poor prognosis (10), and there appears to be an inverse association between loss of CD59 and increased expression of CD55 in malignant tumors (11, 12). In breast carcinomas, however, increased expression of CD46 and lack of CD55 in high-grade tumors has been observed (9). Analysis of CD46 expression in these breast microarrays will complete the picture of the role of these complement inhibitory proteins in tumor prognosis. As there are no commercially available anti-CD46 antibodies, which can be assessed on paraffin sections at the present, we have recently developed a mAb specific to CD46.

Abnormal expression of CD55 may be because of dysregulation of genes that are associated with malignant progression. Alternatively, overexpression of CD55 may be because of selective pressure from complement activation within the tumor environment. Surviving cells may express higher levels of CD55. It may be predicted that these tumors would be more aggressive because they should be resistant to complement attack. However, our study suggests that these tumors are less aggressive. Preliminary gene array studies have suggested that signaling via CD55 on tumors down-regulates inhibitors of apoptosis. Tumors expressing high levels of CD55 would therefore be susceptible to apoptosis and tumors losing CD55 would be resistant. Future studies will test this hypothesis.

Whether abnormal expression of CD55 is because of gene dysregulation or to selective immune pressure remains uncertain. However, our results show that in a series of 480 breast carcinomas, loss of CD55 expression is associated with poor survival.

ACKNOWLEDGMENTS

We thank Robert Moss for technical assistance.

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Loss of CD55 Is Associated with Aggressive Breast Tumors
