Immunostaining patterns of myoepithelial cells in breast lesions: a comparison of CD10 and smooth muscle myosin heavy chain

A N Kalof, D Tam, B Beatty, K Cooper

Background: Recent studies have reported CD10 expression in myoepithelial cells (MEC) of the breast, supporting its use as a marker to help distinguish invasive breast carcinoma (IC) from ductal carcinoma in situ (DCIS).

Aim: To compare the effectiveness of CD10 with smooth muscle myosin heavy chain (SMMHC) in the detection of MEC in benign and malignant breast lesions.

Methods: Histological material from 25 patients with DCIS and 21 with IC were immunostained for CD10 and SMMHC. Staining was scored on a scale of 0 to 3+ (0, no staining; 3+, intense) and the staining distribution was documented as focal, partial, or circumferential.

Results: Uniform, 3+ circumferential CD10 and SMMHC staining of MEC was seen in normal breast ducts and lobules, and in ducts and acini involved in sclerosing adenosis and apocrine metaplasia. In an analysis of total ducts involved by DCIS, 3+ circumferential staining was seen in 65 of 366 ducts (17.7%) stained for CD10 versus 190 of 396 ducts (48%) stained for SMMHC. MEC were not detected immunohistochemically in 116 of 366 ducts (31.7%) with anti-CD10 and 50 of 396 (12.7%) with anti-SMMHC. In contrast, all ICs were negative for both CD10 and SMMHC. Focal background staining of stromal myofibroblasts was seen with both CD10 and SMMHC, but CD10 showed a higher rate of non-specific staining of epithelial cells.

Conclusion: Although CD10 can aid in the distinction between IC and DCIS, SMMHC is a more sensitive and specific marker of MEC and shows less heterogeneity of immunostaining patterns.
the more frequently used SMMHC. We also examined CD10 expression in normal breast tissue and benign lesions, including sclerosing adenosis and apocrine metaplasia.

MATERIALS AND METHODS
Using the SnoMed II system, we searched the Fletcher Allen Health Care surgical pathology archives from July 2000 to June 2003 for excisional breast biopsies with diagnoses of invasive adenocarcinoma (ductal and lobular) and DCIS. Histological material from a total of 31 excisional breast biopsies yielded 25 cases of DCIS and 21 cases of invasive carcinoma (12 ductal and nine lobular), some of which were coexistent. The patients were all female and had a mean age of 57.6 years (range, 32–83). Benign elements were evaluated simultaneously and included three cases of sclerosing adenosis and five cases of apocrine metaplasia. The diagnoses of all patients were confirmed by retrieval of pathology reports and review of all haematoxylin and eosin stained sections by an experienced breast pathologist (DT).

Staining for CD10 was performed using the anti-CD10 monoclonal antibody, clone 56C6 (NCL-CD10-270; NovoCastra, Newcastle upon Tyne, UK). Target retrieval was performed using the Dako (Carpentaria, California, USA) solution of regular sodium citrate, pH 6.0. Application of the primary antibody at a dilution of 1/80 (table 1) was followed by detection using the avidin–biotin–peroxidase complex technique with diaminobenzidine as the chromagen substrate. Staining for SMMHC (M3558; Dako) was performed at a dilution of 1/200. Normal kidney and breast tissues were used as positive tissue controls for CD10, and normal breast tissue was used for SMMHC. Isotype matched murine IgG negative controls were run for each specimen.

MEC, benign and malignant epithelial cells, and stromal myofibroblastic elements were evaluated for percentage of elements staining, staining pattern, and intensity. The staining patterns of the ductal MEC were reported as focal (≤ 10% of duct circumference), partial (10–90%), or circumferential (> 90%). The staining intensity of the various breast elements was evaluated on a scale from 0 to 3+ (0, no staining; 3+, intense staining). The number of ducts involved by DCIS in all 25 cases were totalled and scored. The results of each immunohistochemical study were separated into the following groups: 3+ complete, 3+ partial, 2+ complete, 2+ partial, 2+ focal, 1+ complete, 1+ partial, 1+ focal, and 0.

RESULTS
Table 2 summarises the immunohistochemical results, showing the number of ducts staining positively divided by the total number of ducts involved by DCIS, and the significance of the differences in staining patterns. The following results describe in detail the immunostaining patterns of ducts involved by DCIS analysed by case and by total number of ducts involved.

Ductal carcinoma in situ
Analysis of cases (n = 25)
Staining of the 25 DCIS cases for CD10 revealed six cases with 2+3+ circumferential staining of MEC in all ducts involved by DCIS. Seventeen cases showed heterogeneous staining of the MEC layer: four cases showed weak (0+/+) focal to partial staining of all ducts, five cases showed 2+3+ staining of 10–49% of ducts, four cases showed 2+3+ staining of 50–75% of ducts, and four cases showed 2+3+ staining of 76–99% of ducts. In the remaining two cases MEC were negative for CD10.

Staining of the same cases for SMMHC showed 12 cases with 2+3+ circumferential staining of MEC in all ducts involved by DCIS. Nine cases showed heterogeneous staining of the MEC layer: three cases showed weak (0–1+) focal staining, one case showed 2+3+ staining of 10–49% of ducts, two cases showed 2+3+ staining of 50–75% of ducts, and three cases showed 2+3+ staining of 76–99% of ducts. Four cases showed no staining of MEC for SMMHC, including one case of micropapillary DCIS arising in an intraductal papilloma.

Analysis of total ducts stained for CD10 (n = 366) and SMMHC (n = 396)
Totalling the number of ducts involved by DCIS in all 25 cases, 3+ circumferential CD10 staining was seen in 65 of 366 ducts (17.7%) compared with 190 of 396 ducts (48%) stained for SMMHC. MEC were not detected in 116 of 366 ducts (31.7%) stained for CD10 and were similarly absent in 50 of 396 ducts (12.7%) stained for SMMHC. Within each classification of staining, we compared staining for CD10 with that for SMMHC in the MEC of ducts involved by DCIS. Table 2 shows these results. To determine whether there were significant differences between the two immunohistochemical stains, we conducted Fisher’s exact tests on the underlying 2 × 2 contingency table (percentage positively stained v percentage negatively stained). All differences between tests that were significant had p values of 0.006 or better.

Thus, the statistical analysis demonstrates that SMMHC and CD10 differ with regard to intensity and distribution of MEC staining. The antibody for SMMHC stains a higher proportion of MEC, with 48% of cases showing circumferential staining of the MEC layer in all ducts involved by DCIS, compared with only 24% of cases stained with anti-CD10.

Invasive carcinoma
Staining for both CD10 and SMMHC demonstrated an absence of MEC in all cases of invasive carcinoma (12 ductal and nine lobular). Adjacent blood vessels were strongly reactive for SMMHC, serving as good internal controls. In each of the invasive carcinoma cases, there was focal, 1+ to 2+ patchy background staining of spindled cells for both CD10 and SMMHC (fig 1A–C). These spindled cells were interpreted as myofibroblasts and were associated with the desmoplastic stroma surrounding invasive tumour islands, in addition to the granulation tissue adjacent to previous biopsy sites. The corresponding negative controls did not show immunopositivity of stromal myofibroblasts.

Normal breast elements
Uniform, 3+ circumferential CD10 and SMMHC staining of MEC was seen in normal breast ducts and lobules, in addition to ducts and acini involved in sclerosing adenosis (three of three) and apocrine metaplasia (five of five).

DISCUSSION
Our study found that SMMHC was a better marker than CD10 for the identification of MEC in breast ducts involved by DCIS. Although CD10 was consistently expressed in the MEC of normal breast tissue, sclerosing adenosis, and apocrine metaplasia, it showed a heterogeneous staining pattern in ducts involved by DCIS. Specifically, only 32.7% of ducts stained for CD10 showed complete, strong staining of the MEC layer, and almost a third demonstrated an absence
of staining. However, staining for SMMHC was significantly stronger and more complete in MEC of DCIS. Whereas 61.1% of ducts stained for SMMHC showed complete, 2+ to 3+ staining of the MEC layer, only 12.7% of ducts completely failed to highlight the MEC layer.

Because individual cases showed heterogeneity in MEC staining patterns, which varied considerably between ducts involved by DCIS, the numbers of ducts in all cases were counted and each evaluated individually for intensity and staining pattern. In doing so, we were able to evaluate the characteristics of individual ducts that may have impacted on staining intensity. We found that many ducts that were associated with pronounced inflammation displayed considerably weaker, discontinuous staining for CD10 (fig 2A–C). The discontinuous pattern of staining also raised the possibility of an intermediate or premalignant state, whereby there is a gradual loss of MEC before stromal invasion.

Because the presence of MEC distinguishes benign from malignant disease, it is important that MEC markers do not misinterpret. In our study, CD10 exhibited a reduced staining of background stromal cells but positively staining blood vessels, serving as good internal controls for smooth muscle myosin heavy chain (SMMHC) (staining for SMMHC; original magnification, ×400). Note the positively staining background myofibroblasts (staining for CD10; original magnification, ×400).

Table 2 Immunohistochemical results of DCIS

<table>
<thead>
<tr>
<th>Antigen</th>
<th>3+ complete*</th>
<th>3+ partial</th>
<th>2+ complete*</th>
<th>2+ partial*</th>
<th>1+ complete*</th>
<th>1+ partial*</th>
<th>1+ focal*</th>
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<tr>
<td>CD10</td>
<td>65/366</td>
<td>0/366</td>
<td>55/366</td>
<td>20/366</td>
<td>0/351</td>
<td>29/366</td>
<td>27/366</td>
<td>54/366</td>
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<tr>
<td>SMMHC</td>
<td>190/396</td>
<td>0/379</td>
<td>52/396</td>
<td>52/396</td>
<td>3/396</td>
<td>1/396</td>
<td>13/396</td>
<td>35/396</td>
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<td></td>
<td>(48.0%)</td>
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<td>(13.1%)</td>
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<td>(0.8%)</td>
<td>(0.2%)</td>
<td>(3.3%)</td>
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The values are number of ducts staining positively/total number of ducts involved by DCIS.

*The difference in the proportion of ducts staining positively was significant at p = 0.02 or better using Fisher’s exact test. Other results were not significant at the 0.25 level.

DCIS, ductal carcinoma in situ; SMMHC, smooth muscle myosin heavy chain.

Anti-SMMHC also showed background staining of stromal myofibroblasts, albeit to a lesser degree. Given that myofibroblasts and MEC share a mixed smooth muscle phenotype, antibodies to many markers of smooth muscle differentiation (such as SMA) have shown reduced specificity, with background staining of myofibroblasts. SMMHC has been regarded as a marker of terminal smooth muscle differentiation, and has demonstrated higher specificity for MEC compared with myofibroblasts in many studies.2,19 Positive staining of stromal myofibroblasts for SMMHC has nevertheless been documented previously in breast cancers,21 and our study shows significant crossreactivity with stromal myofibroblasts. Method comparisons show that these studies have used the same SMMS-1 antibody clone with similar dilutions, varying from 1/20 to 1/60.20 We are unable to account for the differences in crossreactivity, indicating that some cases need to be interpreted with caution because the positively staining myofibroblasts abutting infiltrating carcinoma may be misinterpreted as MEC, resulting in a potential misdiagnosis of in situ carcinoma.

“Because no myoepithelial cell marker to date exhibits perfect sensitivity and specificity, it is recommended that a combination of immunohistochemical stains be used when investigating difficult breast lesions.”

Interestingly, a recent study of CD10 expression in colorectal adenomas showed frequent expression of CD10 by stromal cells in adenomas and invasive carcinomas, suggesting that CD10 expression may play an integral part in colorectal carcinogenesis. CD10 has also been detected within invasive areas of breast carcinoma,14,15 supporting its role as a potential participant in the “tumour–stromal interaction.”16 Iwaya et al reported an increased frequency of stromal expression of CD10 in invasive breast carcinoma cases with axillary lymph node metastases.17 In addition, they correlated stromal CD10 positivity with a shorter time to recurrence, thereby suggesting that stromal expression of CD10 may serve as an important prognostic indicator, a function that would preclude its use as an ideal MEC marker because of interfering background staining.18

Figure 1  (A) Invasive ductal adenocarcinoma (haematoxylin and eosin stain; original magnification, ×400). (B) Invasive ductal adenocarcinoma. Note the positively staining background myofibroblasts (staining for CD10; original magnification, ×400). (C) Invasive ductal adenocarcinoma. Note the reduced staining of background stromal cells but positively staining blood vessels, serving as good internal controls for smooth muscle myosin heavy chain (SMMHC) (staining for SMMHC; original magnification, ×400).

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There are other factors suggesting that staining for SMMHC is superior to staining for CD10 for the identification of MEC in breast samples. For example, similar to other studies, we found that optimum staining for CD10 required the antibody to be diluted 1:80 compared with 1:200 for SMMHC. In addition, there is a considerable cost differential between the two antibodies (in our case, $360/ml for anti-CD10 v $240/ml for anti-SMMHC), indicating that SMMHC is more cost effective for routine use in the laboratory.

More sensitive and specific markers of MEC are constantly being sought to help pathologists with difficult breast lesions. P63, a recently identified member of the p53 gene family, has been found to be another reliable and sensitive marker of MEC of the breast.29-32 Of particular interest, Barbasso et al showed that the background myofibroblastic cells were consistently non-reactive with anti-p63.33 A more recent study by Werling et al compared the usefulness of p63 with calponin and SMMHC for identifying MEC in breast tissue. They found that although p63 offers excellent sensitivity and increased specificity for MEC, the antibody to p63 reacted with a small proportion (11%) of breast carcinoma tumour cells. In addition, anti-p63 occasionally demonstrated a discontinuous MEC layer surrounding nests of DCIS. Werling et al recommend that expression of p63 should be used in conjunction with SMMHC for the identification of MEC in difficult breast lesions.34

In conclusion, although expression of CD10 may aid in the distinction between DCIS and invasive breast carcinoma, SMMHC exhibits increased sensitivity and specificity for MEC and is more cost effective for routine use in identification of MECs of the breast. We have found that staining for SMMHC has worked well in our laboratory and use it routinely in isolation for the detection of breast MEC. However, because no MEC marker to date exhibits perfect sensitivity and specificity, it is recommended that a combination of immunohistochemical stains be used when investigating difficult breast lesions.

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REFERENCES

Take home messages
- Although CD10 expression can aid in the distinction between invasive breast carcinoma and ductal carcinoma in situ, smooth muscle myosin heavy chain (SMMHC) is a more sensitive and specific marker of myoepithelial cells and shows less heterogeneity of immunostaining patterns.
- In addition, staining for SMMHC is more cost effective than staining for CD10.
- Because none of the myoepithelial markers shows perfect sensitivity and specificity, a combination of immunohistochemical stains should be used when investigating difficult breast lesions.

Figure 2  (A) Focus of ductal carcinoma in situ (DCIS) with surrounding inflammation (haematoxylin and eosin stain; original magnification, ×200). (B) Immunohistochemistry for CD10 on the same focus of DCIS. Note the lack of a positively staining myoepithelial cell (MEC) layer (CD10; original magnification, ×200). (C) Immunohistochemistry for smooth muscle myosin heavy chain (SMMHC) on the same focus of DCIS with strong staining of the MEC layer (SMMHC; original magnification, ×200).
CD10, SMMHC, and breast myoepithelial cells

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