CK5 Is More Sensitive Than CK5/6 in Identifying the “Basal-like” Phenotype of Breast Carcinoma

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Abstract

Multiple immunohistochemical stains, including cytokeratin (CK)5/6, are used in a panel format to identify “basal-like” carcinomas. We set out to determine the sensitivity and specificity of the CK5 antibody (clone XM26) and compared its expression with that of CK5/6 (clone D5/16B4) in a variety of breast carcinoma cases. The study was performed on 3 breast carcinoma tissue microarrays (TMAs). TMA-1 consisted of 59 consecutive breast carcinoma cases. TMA-2 (n = 16) and TMA-3 (n = 11) consisted of basal-like breast carcinomas previously characterized morphologically and immunohistochemically at our institution. Of the 86 total cases, 20 were positive for CK5 and CK5/6, 14 were positive for CK5 only, and 52 were negative for both. The sensitivity of CK5 for identifying basal-like tumors was 97% compared with 59% for CK5/6. Both antibodies had comparable specificity of more than 95%. For positive cases, the percentage and intensity of staining was much higher with CK5 than with CK5/6 (P = .0001).

Gene expression profiling studies have identified various breast carcinoma classes with prognostic significance.¹,² The 5 distinct classes identified by expression profiling include luminal A, luminal B, ERBB2, normal breast-like, and “basal-like.” The luminal tumors are hormone receptor–positive and negative for HER2 and tend to have a good prognosis. In contrast, ERBB2 tumors are hormone receptor–negative and positive for HER2 and have been shown to have poor prognosis. Normal breast-like tumors express genes characteristic of adipose tissue and other nonepithelial breast elements and have a relatively poor prognosis. Among all of the molecular classes, basal-like breast carcinoma seems to have the worst prognosis.³ These tumors are hormone receptor–negative and HER2-negative. Basal-like tumors are also the most common type of tumors in patients with germline BRCA1 mutations.³-⁶

The morphologic features and immunophenotype of these basal-like tumors have been recently described.⁷,⁸ They are high-grade and generally circumscribed and often have a lymphoplasmacytic inflammatory infiltrate. The immunohistochemical markers often used for identifying basal-like tumors include cytokeratin (CK)5/6, CK14, CK17, epidermal growth factor receptor (EGFR), vimentin, and p63.⁷,⁹ The expression of these markers in a tumor with basal-like morphologic features can range from focal, weak staining to diffuse, strong reactivity.¹⁰ The sensitivity of these markers is high. Basal markers reported in the literature based on direct correlation with expression profiling is shown in Table I.⁷,⁸,¹¹ Although some markers (vimentin and EGFR) seem to be very sensitive, they are not entirely specific for the basal-like phenotype.

There is currently some debate in the literature whether triple-negative (negative for hormone receptors and HER2) tumors equate with basal-like carcinoma.¹²,¹³ A recent study

CME

Upon completion of this activity you will be able to:
- list the molecular classes of breast carcinoma.
- differentiate molecular classes of breast carcinoma using selected immunohistochemical markers.
- compare the utility of CK5 and CK5/6 antibodies in identifying the “basal-like” subtype of breast carcinoma.

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Materials and Methods

The study was performed on 3 breast carcinoma tissue microarrays (TMAs). The TMAs were manually constructed using the Beecher Instruments (Sun Prairie, WI) MTA-I machine. Each tumor was represented by 3 cores (3-fold redundancy). Each core diameter was 0.6 mm. The details of tumors represented on each TMA are as follows: TMA-1 consisted of 11 cases of consecutive breast carcinomas. Of these 11 cases, 48 were ductal carcinomas (including 8 tumors showing basal-like morphologic features), 8 lobular carcinomas, 2 mixed ductal and lobular carcinomas, and 1 metaplastic carcinoma (mixed adenocarcinoma and chondrosarcoma components; TMA contained only the adenocarcinoma component). TMA-2 consisted of 16 basal-like breast carcinomas in women younger than 40 years. TMA-3 consisted of 11 basal-like breast carcinomas in women 40 years or older. The basal-like carcinomas represented on TMA-2 and TMA-3 have been previously characterized at our institution. They were all high-grade, triple-negative tumors with characteristic basal-like morphologic features. These tumors have been subjected to a panel of immunohistochemical stains known for identifying basal-like carcinomas (CK5/6, CK14, CK17, and EGFR), and all have shown some staining for at least 2 of the 4 aforementioned markers.

We immunostained 4-µm TMA sections on the Benchmark XT automated stainer (Ventana Medical Systems, Tucson, AZ). The protocol consisted of a pretreatment with CC1, pH 8.0 (Ventana), followed by incubation with CK5 (clone XM26, dilution 1:25; Novocastra-Vision Biosystems, Norwell, MA) or CK5/6 (clones D5 and 16B4, prediluted; Ventana) mouse monoclonal antibodies. The antigen-antibody complexes were detected by using an iVIEW DAB detection kit (Ventana).

A semiquantitative scoring method (modified from the previously described H-score method) was used to report scores for immunohistochemical stains in the present study. This method takes into account percentage and intensity of staining. The percentage of positive cells is multiplied by the intensity of staining (0, 1+, 2+, or 3+), followed by addition of all values. Therefore, the score ranges from 0 through 300. A score of 10 or less was considered negative.

The sensitivity and specificity for CK5 and CK5/6 were manually calculated. Further statistical analysis was performed using the Arcus Quickstat software program (Longman Software Publishing, Cambridge, England). The difference in staining of basal-like tumors (TMA-2 and TMA-3 cases) between CK5 and CK5/6 stains was assessed by using the \( \chi^2 \) test. The difference of the mean immunohistochemical scores between CK5 and CK5/6 was assessed by using the Student \( t \) test. Statistical significance was defined as a \( P \) value less than .05.

Results

Overall, the sensitivity of CK5 for detecting the basal-like phenotype was 97% compared with 59% for CK5/6. The specificity for CK5 was 96% compared with 98% for CK5/6 for identifying basal-like carcinomas (CK5/6, CK14, CK17, and EGFR), and all have shown some staining for at least 2 of the 4 aforementioned markers.

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Details of TMA-1 Cases

Of the 59 cases of consecutive breast carcinomas, 52 were negative for CK5 and CK5/6. Five cases were positive for CK5 and CK5/6, and 2 cases were positive only for CK5. All 7 positive cases by CK5 were identified as basal-like by morphologic studies. Of these 7 cases, 5 were triple negative (estrogen receptor [ER]–, progesterone receptor [PR]–, and HER2–), and 2 showed

Table 1

Sensitivity of Immunohistochemical Markers in Identifying Basal-like Carcinomas

<table>
<thead>
<tr>
<th>Marker/Reference</th>
<th>No. of Cases Studied</th>
<th>Sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK5/6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nielsen et al²</td>
<td>21</td>
<td>62</td>
</tr>
<tr>
<td>Livasy et al²</td>
<td>18</td>
<td>61</td>
</tr>
<tr>
<td>Lakhani et al¹¹*</td>
<td>158</td>
<td>57.6</td>
</tr>
<tr>
<td>EGFR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nielsen et al²</td>
<td>21</td>
<td>57</td>
</tr>
<tr>
<td>Livasy et al²</td>
<td>18</td>
<td>72</td>
</tr>
<tr>
<td>Lakhani et al¹¹*</td>
<td>158</td>
<td>67.3</td>
</tr>
<tr>
<td>Vimentin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Livasy et al²</td>
<td>18</td>
<td>94</td>
</tr>
<tr>
<td>p63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Livasy et al²</td>
<td>18</td>
<td>22</td>
</tr>
<tr>
<td>CK14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lakhani et al¹¹*</td>
<td>158</td>
<td>60.6</td>
</tr>
<tr>
<td>CK17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lakhani et al¹¹*</td>
<td>158</td>
<td>53.3</td>
</tr>
</tbody>
</table>

CK, cytokeratin; EGFR, epidermal growth factor receptor.

* Study performed on tumors from BRCA1 mutation carriers.

by Cheang et al¹⁴ has shown that in triple-negative tumors treated with adjuvant anthracycline-based chemotherapy, the additional positive basal markers identify a cohort of patients with a significantly worse outcome. However, our aim was to determine the sensitivity and specificity of the CK5 antibody and compare its usefulness with the most commonly used marker of basal-like tumors, CK5/6.
some ER (immunohistochemical score, 20) and PR (immunohistochemical score, 90) staining. For all positive cases, the percentage and intensity of staining was much higher with CK5 than with CK5/6 (see CK5 and CK5/6 scores) Table 4. Of all 52 negative cases, only 2 had an unexpected result. One basal-like breast carcinoma (by morphologic studies) was negative for CK5 and CK5/6. However, in this case only 1 of 3 cores had substantial tumor and may have been falsely negative. The other unexpected result was for metaplastic carcinoma. Recent studies have indicated that metaplastic carcinoma also expresses basal-like CKs. This case was a mix of adenocarcinoma and a chondrosarcomatous component. The TMA cores contained only adenocarcinoma. Because basal-type CKs are more often positive in spindle cell metaplastic carcinoma, the absence of a spindle cell component in this case may explain the negative result.

**Comparison of CK5 with CK5/6 for Staining Basal-like Carcinomas (TMA-2 and TMA-3)**

At an immunohistochemical score cutoff of 10, all 27 basal-like breast carcinomas were positive for CK5. In contrast, CK5/6 staining was seen only in 15 (56%) of 27 tumors. The difference between CK5 and CK5/6 staining was statistically significant ($P = .0001$). The percentage and intensity of staining was much higher with CK5 than with CK5/6 Image 11, Image 21, and Image 31. The details of CK5 and CK5/6 scores are provided in Table 5. The difference in mean immunohistochemical scores between CK5 and CK5/6 (for all basal-like tumors) was 149.3 and was statistically significant ($P = .0001$). The difference in mean immunohistochemical scores between CK5 and CK5/6 (for CK5+ and CK5/6+ cases only) was 168.6 and was also statistically significant ($P = .0001$).

**Discussion**

This is the only study that directly compares the sensitivity and specificity of CK5 antibody, clone XM26 (Vision Biosystems), with a CK5/6 cocktail, clones D5 and 16B4 (Ventana). These antibodies are typically used as part of a panel to identify basal-like breast carcinoma.

Several studies have demonstrated that an efficient panel of antibodies for detecting the basal-like variant among triple-negative (ER, PR, and HER2) carcinomas is CK5/6, EGFR, CK14, CK17, and vimentin. The actual proportion of cells and the precise expression of these antigens are not well defined in the literature. Most studies cite that the basal variant expresses 2 or more of these antigens in a patchy manner or in a strong, diffuse pattern. However, it must be emphasized that the morphologic picture of the lesion is a critical component of the examination that leads to the categorization.
of the basal-like variant. These tumors are well circumscribed and often have lymphoid infiltrates, a solid growth pattern, a Nottingham score of 8 or 9, and a nuclear grade of 3, in addition to the triple negative status for ER, PR, and HER2. Cheang et al recently demonstrated that in triple-negative tumors treated with adjuvant anthracycline-based chemotherapy, the additional positive basal markers identify a cohort of patients with a significantly worse outcome. Therefore, identifying the basal phenotype by immunohistochemical staining in triple-negative tumors is of clinical significance.

In our well-characterized group of basal-like tumors, we demonstrated that the CK5 antibody was positive (all cases) in 44% more basal-like tumors than was demonstrated with the antibody to CK5/6 (15/27 cases). As can be seen in Table 5, the cases that were positive with CK5 were substantially positive (mean score, 125) compared with the negative CK5/6 results. The specificity of the CK5 was demonstrated on TMA-1, as it showed expression similar to CK5/6 for ductal carcinomas of other nonbasal phenotypes. All 3 TMAs also demonstrated a substantial increase in the intensity of immunostaining for CK5 compared with CK5/6 for all tumors. We emphasize that for the detection of the basal-like breast carcinoma phenotype, we found the CK5 antibody to be a more sensitive keratin antibody than the CK5/6 antibody, and they have comparable specificity.

In our series of basal-like tumors represented on TMA-2 and TMA-3, CK5 was positive in all cases. However, these cases have selection bias among triple-negative tumors because they have been previously positive for at least 2 of the 4 routinely used basal markers (CK5/6, CK14, CK17, and CK19).
We chose to study this data set because this is the only definitive way of determining CK5 sensitivity in lieu of expression profiling. Although a few triple-negative tumors not previously analyzed for other basal markers (5 cases in TMA-1) were evaluated for CK5, the triple-negative tumors with confirmed negativity for all 4 basal markers (CK5/6, CK14, CK17, and EGFR) were not assessed for CK5 in this study. If a large number of triple-negative tumors are evaluated with CK5, it is conceivable that some tumors will still be negative for CK5 and will be classified as triple-negative, nonbasal type. According to Cheang et al,14 there is additional value of basal-like markers in triple-negative tumors in identifying a cohort of patients with a worse outcome. However, they used CK5/6 and EGFR for defining their core basal group within triple-negative tumors. It will be of interest to know if their findings could be reproduced or better defined by using the CK5 antibody.

Historically, when Moll et al19 described the expression pattern of CKs in human tissue, it was stated that “polypeptide components 4-6 were observed in various proportion in many non-stratified squamous epithelia, as well as epithelia of trachea and apocrine and sweat glands of the skin and mammary gland.” Furthermore, it was also stated that “polypeptides 5 and 6 also occur in the epidermis and hair follicles and are closely related to each other.” Both CK5 and CK6 are basic (type II) polypeptides with molecular weights of 58 and 56 kDa, respectively.20

Given the historic data and similar expression of CK5 and CK6, the higher sensitivity of CK5 antibody is somewhat difficult to explain. The immunogen for the CK5 antibody
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An anatomic pathology study was conducted using a prokaryotic recombinant fusion protein corresponding to a 103-amino-acid portion of the C-terminal region of the human CK5 molecule. The CK5/6 antibody (clone D5/16B4) is a cocktail of mouse monoclonal antibodies raised against purified CK proteins and shows reactivity against the CK5 and CK6 proteins. It appears that the 2 antibodies recognize different epitopes, and the affinity of CK5 to its antigenic determinant is probably greater to that of CK5/6 and likely explains the higher sensitivity of CK5.

Another possible reason for the lower sensitivity of CK5/6 could be that antibodies (CK5 and CK6) interfere with each other’s binding by steric hindrance. Moreover, an in-depth analysis of breast cancer gene expression data shows that basal-like breast cancers demonstrate increased expression only for CK5, and there is a lack of evidence for CK6 messenger RNA expression in normal breast and basal-like breast carcinomas.

The CK5/6 antibody has found wide use in tumor pathology, especially for distinguishing mesothelioma from pulmonary adenocarcinoma and its preferential immuno-staining of squamous and transitional cell carcinomas. We have not studied the CK5 antibody profiles of these tumors, and similar comparative studies are warranted to determine whether CK5 is superior to CK5/6 in these distinctions.

Because of its superior sensitivity, we recommend the use of the CK5 antibody in the panel workup of breast carcinomas with a basal-like phenotype.

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References