Cytokeratin 8 Immunostaining Pattern and E-Cadherin Expression Distinguish Lobular From Ductal Breast Carcinoma

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Abstract

Immunohistochemistry using antibodies to cytokeratin 8 can serve as a valuable diagnostic tool for the differentiation of lobular from ductal carcinomas of the breast. In contrast with ductal carcinomas, which exhibit a peripheral-predominant immunostaining pattern, adjacent tumor cells “molding” to each other, lobular carcinomas exhibit a ring-like perinuclear immunostaining pattern, creating a “bag of marbles” appearance with neighboring tumor cells. This immunostaining pattern is stable even in the tumors that otherwise do not exhibit characteristic histomorphologic features (ie, solid or pleomorphic type of a lobular carcinoma) and tumors that mimic growth patterns characteristic of the respective other tumor type (ie, targetoid or single-file growth pattern in a ductal carcinoma). Furthermore, we demonstrate that ductal carcinomas express E-cadherin in a similar peripheral-predominant immunostaining pattern (33/33 cases), while all 15 lobular carcinomas were negative for E-cadherin, suggesting a role for E-cadherin in the architectural organization of the cytoskeletal scaffolding within the tumor cells.

Infiltrating lobular carcinoma of the breast, which accounts for roughly 10% to 15% of breast cancer cases, is distinguished histologically from infiltrating ductal carcinoma by its characteristic small cells with inconspicuous nucleoli. Lobular carcinomas demonstrate a different infiltrative pattern, typically as linear arrays (files) or single cells, and classically forming targetoid patterns around nonneoplastic ducts. Infiltration typically occurs in a manner that does not destroy anatomic structures or excite a substantial connective tissue response. Pure lobular carcinomas have been associated with a prolonged disease-free survival compared with cases of infiltrating ductal carcinoma, in particular during the early stages of the disease. However, a number of variants of invasive lobular carcinoma have been described, including solid, alveolar, mixed, histiocytoid, tubulolobular, and pleomorphic types, the latter exhibiting moderate to high nuclear grades. Besides their characteristic histologic features, these variants are characterized by an increased risk of recurrence and an overall less favorable prognosis than classic lobular carcinoma.

By virtue of their distinct growth pattern, lobular carcinomas often fail to form distinct masses that can be diagnosed by palpation or mammography. This makes early diagnosis difficult and jeopardizes the results of surgical treatment. Since tumor cells tend to infiltrate beyond the palpable extent of the tumor, resection margins are more frequently tumor positive and in-breast recurrence rates are higher than in ductal carcinomas.

Despite efforts to obtain wide local resection margins, lobular carcinomas treated with lobectomy require conversion into mastectomy more than 2 times more frequently than ductal carcinomas. Also, lobular
carcinomas follow different metastatic pathways compared with ductal carcinomas, preferentially metastasizing into bone marrow, endocrine organs, meninges, and peritoneal or retroperitoneal sites.\textsuperscript{12-14} Of special importance for the treatment and follow-up of patients with breast cancer is the fact that infiltrating lobular carcinomas have a substantially increased propensity for multifocal (multiple tumor sites within 1 quadrant) and multicentric (multiple tumor sites within 1 breast) distribution, and for bilaterality (tumor sites within the contralateral breast).\textsuperscript{15-18} Particular attention should be given to the histologic examination of axillary nodes in resection specimens of lobular carcinoma since node metastases are much more often missed and false-negative results reported compared with ductal carcinomas.\textsuperscript{19}

Since most of the differences between lobular and ductal carcinomas affect the diagnostic and therapeutic management for patients with breast cancer, as well as adequate follow-up, the differentiation of lobular from ductal carcinomas is of exquisite importance in a surgical pathology report. In most cases, this can be accomplished because of distinct histomorphologic features. However, occasionally, this task may become quite difficult, in particular when dealing with the variants of infiltrating lobular carcinoma. Since both lobular and ductal carcinomas originate from the same cell type, the terminal duct lobular unit,\textsuperscript{5} conventional immunohistochemistry using cell type–specific antibodies offers little help. However, we demonstrate that the immunohistochemical staining pattern of antibodies against the low-molecular-weight cytokeratin 8 and to E-cadherin offer valuable diagnostic tools for the distinction between the 2 tumor subtypes.

**Materials and Methods**

Immunohistochemical studies were performed according to previously published protocols.\textsuperscript{20-22} Briefly, 4- to 5-µm sections from representative blocks in each case were deparaffinized, rehydrated in graded alcohols, and subjected to heat-induced epitope retrieval in a 0.1-mol/L concentration of citrate buffer (pH 6) using a microwave oven for 8 minutes.\textsuperscript{22} Sections then were incubated with the primary antibodies **Table 1** for 45 minutes at room temperature. Localization was performed via the standard avidin-biotin immunoperoxidase method\textsuperscript{20} with nickel chloride 3,3′-diaminobenzidine as the chromogen.\textsuperscript{21} Sections then were counterstained with hematoxylin or methyl green. Appropriate positive and negative controls were included with each immunostaining procedure. Routine H&E staining was performed according to standard protocols.

### Table 1

**Antibodies Used**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Working Dilution</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokeratin 8</td>
<td>CAM5.2</td>
<td>1:50</td>
<td>Becton Dickinson, Mountain View, CA</td>
</tr>
<tr>
<td>Cytokeratin 8</td>
<td>35BH11</td>
<td>1:2000</td>
<td>Zymed, San Francisco, CA</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>67A4</td>
<td>1:200</td>
<td>Zymed, San Francisco, CA</td>
</tr>
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</table>

**Results**

**Cytokeratin 8 Immunostaining Pattern**

We found a distinct difference in the cytokeratin 8 immunostaining patterns in ductal carcinomas as compared with lobular carcinomas. In ductal carcinomas, chromogen distribution was in a diffuse cytoplasmic pattern **Image 1A** and **Image 1B**. In contrast, chromogen distribution was in a ring-like, perinuclear distribution in lobular carcinomas **Image 1C** and **Image 1D**. While ductal carcinoma cells showed molding of the cytokeratin-associated chromogen at the border to neighboring tumor cells, forming solid tumor cell arrangements (Image 1B), no such molding was observed in lobular carcinomas (Image 1D). Rather, an impression of a “bag of marbles” was created by the spatial arrangement of the chromogen rings around the nucleus (Image 1D). A similar difference between ductal and lobular carcinoma also was seen in the in situ lesions, with ductal in situ carcinoma cells exhibiting cytoplasmic and peripheral-predominant “molding” chromogen patterns **Image 2A** and lobular in situ lesions creating the impression of a bag of marbles **Image 2B**. The difference in cytokeratin immunostaining pattern between the 2 cells types is emphasized further in the pagetoid growth of lobular carcinoma (ring-like immunostaining pattern) undermining nonneoplastic ductal epithelium (peripheral-predominant, molding pattern) **Image 3**.

Occasionally, ductal carcinomas mimic the single-file infiltrative growth pattern (Image 1A) that usually is seen in lobular carcinoma (Image 1C), but the distinctive cytokeratin immunostaining pattern helps to distinguish between the 2 cancer subtypes (Images 1B and 1D). Likewise, ductal carcinomas may mimic the targetoid infiltrative pattern usually seen in lobular carcinomas, but the cytokeratin immunostaining pattern leaves little room for misinterpretation **Image 4**. The characteristic immunostaining pattern also is illustrated in a case of a tumor that exhibited both ductal and lobular features **Image 5**. Note that the 2 distinct cancer cell types are virtually identical in routine H&E staining (Image 5A) but exhibit distinct anti-cytokeratin immunostaining patterns.
E-Cadherin Immunohistochemical Results

Of the 33 ductal breast cancers, all showed E-cadherin expression in a similar peripheral-predominant membranous pattern seen in the cytokeratin 8–immunostaining pattern of ductal carcinomas (not shown). In contrast, all 15 tested lobular carcinomas were negative for E-cadherin expression by immunohistochemistry.

Transmission Electron Microscopy

In 10 selected cases of lobular carcinomas, we observed the perinuclear accumulation of intermediate filaments in thick bundles Image 6I. Similar filament bundles were not observed in 10 cases of ductal breast carcinoma.

Discussion

We demonstrated that the pattern of anti–cytokeratin 8 immunostaining can help to distinguish between lobular and ductal carcinomas. While ductal carcinomas show a characteristic cytoplasmic peripheral-predominant pattern in which neighboring cells tend to mold together into cohesive tumor cell groups (Image 1B), lobular carcinomas
show a perinuclear ring-like immunostaining pattern, virtually “ignoring” the neighboring cells (Image 1D). This way, an arrangement of in situ or infiltrating tumor cells shows an immunostaining pattern reminiscent of grapes or a bag of marbles (Images 1D and 2B). This is best documented when ductal and lobular cells are immediately side by side, as in the case of a tumor exhibiting 2 distinct cell types (Image 5) or in a case of pagetoid spread of a lobular carcinoma under normal ductal epithelial cells (Image 3). The characteristic immunostaining pattern is maintained even in cases of aberrant architectural cellular arrangement, such as a ductal carcinoma growing in single files (Image 1B) or exhibiting a targetoid growth pattern (Image 4).

The ring-like immunostaining pattern for cytokeratin 8 in lobular carcinoma is reminiscent of the ring-like cytokeratin immunostaining pattern in mesothelioma.\(^23\) This immunostaining pattern in mesothelioma was described as perinuclear, compared with adenocarcinomas that were described as peripheral-predominant.\(^23\) We have adopted these terms for the description of the immunostaining patterns in lobular and ductal carcinomas, respectively.

Cytokeratins are intermediate filaments that form a complex cytoskeleton network within cells and interact with the cytoskeleton of neighboring cells. For this purpose, cytokeratin filaments are anchored to the cell surface via binding to desmosomal proteins and, thus, mediate the interaction between neighboring cells, providing mechanical stability to groups of cells.\(^24,25\) The membrane-predominant immunostaining pattern in cohesive ductal carcinoma and the perinuclear (nonmembranous) pattern in dyscohesive lobular carcinoma points toward a loss or defect of cytoskeletal cell-cell interaction in lobular carcinoma. Electron microscopic studies of lobular carcinoma cells regularly demonstrate dense filament bundles in a perinuclear location (Image 6). Similar bundles of cytokeratin filaments had been pointed out as a characteristic finding in lobular carcinoma.\(^26\) Also, Steinbrecher and Silverberg\(^27\) noted fibrils in a perinuclear
arrangement in electron micrographs of lobular carcinoma cells, and Nesland and coworkers28 demonstrated thick bundles of filaments in circular arrangements within the cytoplasm of lobular carcinoma cells. The loss of cytoskeletal interaction with the cell surface and the molecules involved in cell-cell interaction could provide the biologic basis for the characteristic filing and single cell pattern of infiltration of lobular carcinoma (Images 1C and 1D).

Another cell-cell adhesive mechanism frequently is defective in lobular carcinoma: the E-cadherin cell adhesion system involved in cell-cell interaction through coupling of cytoskeleton-associated desmosomal proteins. E-cadherin immunostaining shows a membrane-predominant immunostaining pattern in ductal carcinomas but frequently is lost in lobular carcinomas and other malignant tumors.29 In our series, 33 of 33 ductal carcinomas were E-cadherin positive (100%), but not a single case of 15 lobular carcinomas showed evidence of E-cadherin expression by immunohistochemistry (0%). This finding is well in keeping with previous reports on a distinct loss of functional E-cadherin
expression in lobular carcinoma, due to loss of messenger RNA or due to mutations in the extracellular portion of the cadherin protein. Indeed, the loss of E-cadherin was associated with tumor differentiation and with invasiveness and lymph node metastases. Based on the observation reported in the present article and the fact that E-cadherin is critical for the anchoring of cytokeratin filaments to the cell surface and the formation of effective cell-cell adhesions, we suggest that the loss of E-cadherin is linked directly with the “collapse” of intermediate filaments within the cytoskeleton and their perinuclear accumulation and, thus, may account for the characteristic perinuclear ring-like cytokeratin 8 immunostaining pattern.

We present cytokeratin 8 and E-cadherin immunohistochemical studies as effective auxiliary tools for the distinction between lobular and ductal carcinomas in the routine diagnosis of breast cancer.

References


