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Introduction

Infiltrating ductal carcinoma is the most common histologic phenotype of breast cancer, comprising 70% to 80% of all cases. The diagnostic distinction between ductal carcinoma in situ and lobular carcinoma in situ of the breast is critical in therapeutic planning. The treatment for ductal carcinoma in situ typically focuses on eradication of the lesion, requiring an accurate assessment of tumor margins, followed by radiation therapy. Lobular carcinoma in situ, however, is considered to put the patient at increased risk for future invasive disease and it is typically not treated surgically. This difference in treatment strategy requires accurate discrimination between lesions of ductal carcinoma in situ and lobular carcinoma in situ.

Immunohistochemical studies have shown E-cadherin to be expressed in breast ductal carcinoma with loss of expression in lobular carcinoma. As a result, mouse monoclonal anti-E-cadherin [HECD-1] has been used by pathologists to differentiate between ductal and lobular carcinomas of the breast, with currently published sensitivity and specificity of approximately 90%.

Rabbit monoclonal antibodies have been shown to be superior to certain mouse monoclonals, and may combine the best properties of both mouse monoclonal antibodies and rabbit antisera. This study compared the staining intensity and sensitivity of a new rabbit monoclonal E-cadherin [EP700Y] vs. a mouse monoclonal E-cadherin [HECD-1] on invasive ductal and lobular breast carcinoma specimens. The two E-cadherin antibodies were further applied in the discrimination of ductal from lobular carcinomas, in combination with a p120 catenin antibody (LC/DC Breast Cocktail).

Methods

Rabbit monoclonal E-cadherin [EP700Y] (Biocare Medical) and mouse monoclonal E-cadherin [HECD-1] (Biocare Medical) were titered and optimized for immunohistochemical staining in infiltrating ductal carcinoma using an HRP-polymer detection system and visualization with DAB. We evaluated 81 cases of infiltrating ductal carcinoma and 28 cases of lobular carcinoma.

A double-stain procedure was performed using a primary antibody cocktail of rabbit monoclonal E-cadherin [EP700Y] and mouse monoclonal p120 [98/pp120] (Biocare Medical), followed by an anti-rabbit HRP and anti-mouse AP detection system, and visualization with DAB and Fast Red, respectively.

For each antibody, cases were considered “positive” if more than 5% of tumor cells were stained. Conversely, cases with less than 5% of tumor cells staining were considered “negative”. A semi-quantitative scoring system for intensity of membrane staining with E-cadherin was applied: 0=negative, 1=weak, 2=medium, 3=strong to intensely strong.

The rabbit monoclonal E-cadherin and mouse monoclonal p120 catenin antibodies were also combined in an antibody cocktail for double stain evaluation.

Results

The rabbit monoclonal E-cadherin antibody showed increased staining sensitivity (99%, 80/81) when compared to mouse monoclonal HECD-1 (93%, 75/81) (Table 1); however, statistical significance was not reached for this comparison (p=0.074).

The rabbit E-cadherin antibody exhibited stronger staining intensity than mouse E-cadherin, as indicated by the average staining scores of 2.8 achieved by rabbit E-cadherin vs. 1.7 by mouse E-cadherin (Table 2). While specificity for the 2 antibodies was comparable, in all cases, rabbit E-cadherin provided darker staining than the mouse E-cadherin. The majority of cases were scored 3+ when stained with rabbit E-cadherin, as compared to scores of 1+ or 2+ for most cases when stained with mouse E-cadherin. Of the 81 breast ductal carcinoma cases, 69 cases had a score of 3+ when stained with rabbit E-cadherin; whereas, only 17 cases had a score of 3+ with mouse E-cadherin. Furthermore, even cases staining 3+ with both rabbit and mouse E-cadherin, consistently demonstrated more intense staining with rabbit E-cadherin. Figures 1-3 show comparisons of rabbit and mouse E-cadherin staining the same ductal carcinoma specimen and demonstrates the greater staining intensity of rabbit E-cadherin.

For example, a specimen staining with rabbit E-cadherin had a score of 2, while the staining of mouse E-cadherin was negative (Figure 1). The specimens in Figures 2 displayed the staining at one score higher with rabbit E-cadherin than with mouse E-cadherin.
Results Continued
Figure 3 shows a specimen that exhibited a staining score of 3 with both rabbit and mouse E-cadherins; however, rabbit E-cadherin provided a darker and more vibrant stain than mouse E-cadherin.

When rabbit E-cadherin was evaluated in combination with p120 catenin in a double-stain procedure, no staining of rabbit E-cadherin was observed in any tumor cells of lobular carcinoma cases. All lobular carcinomas were confirmed by the diffuse cytoplasmic expression of p120 catenin. Figure 4 shows p120 + E-cadherin staining in invasive lobular carcinoma (Figure 4A), lobular carcinoma in situ (LCIS) (Figure 4B), and normal breast ducts (Figure 4C). Rabbit E-cadherin only stained normal breast ducts and did not stain lobular carcinoma (Figures 4A-C).

Discussion
The rabbit E-cadherin [EP700Y] provided a higher sensitivity in all cases vs. clone HECD-1. In some cases, rabbit E-cadherin stained necrotic tissues and/or dead tumor cells, while HECD-1 was negative; however the overall stronger staining of infiltrating ductal carcinomas was easier to interpret, especially in cases where low or negative staining was observed with mouse E-cadherin. This was especially true when evaluating E-cadherin and p120 double stains. E-cadherin is a negative stain in lobular carcinomas, and in difficult cases, especially in cases where microinvasive lobular carcinoma may be present, false negatives could occur. By combining both stains on a single section, not only was limited tissue conserved, but both breast phenotypes can be observed simultaneously. Only E-cadherin membrane staining was observed in infiltrating ductal carcinomas; and lobular carcinomas were E-cadherin negative and p120 positive (Figures 4A-C).

Figures
Table 1: Staining Sensitivity of Rabbit Monoclonal E-cadherin [EP700Y] and Mouse Monoclonal [HECD-1] in Infiltrating Breast Ductal Carcinoma

<table>
<thead>
<tr>
<th>Antibody</th>
<th># of cases</th>
<th>Positive</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit E-cadherin [EP700Y]</td>
<td>81</td>
<td>80</td>
<td>99%</td>
</tr>
<tr>
<td>Mouse E-cadherin [HECD-1]</td>
<td>81</td>
<td>75</td>
<td>93%</td>
</tr>
</tbody>
</table>

Table 2: Intensity of Membrane Staining between Rabbit and Mouse Monoclonal E-cadherin in Infiltrating Breast Ductal Carcinoma

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Score 0</th>
<th>Score 1</th>
<th>Score 2</th>
<th>Score 3</th>
<th># of cases</th>
<th>Average score</th>
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</thead>
<tbody>
<tr>
<td>Rabbit E-cadherin</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>69</td>
<td>81</td>
<td>2.8</td>
</tr>
<tr>
<td>Mouse E-cadherin</td>
<td>6</td>
<td>27</td>
<td>31</td>
<td>17</td>
<td>81</td>
<td>1.7</td>
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</tbody>
</table>

Conclusion

The high affinity rabbit monoclonal E-cadherin antibody [EP700Y] demonstrated higher sensitivity and more intense staining than clone HECD-1 in ductal carcinomas. Moreover, the rabbit monoclonal E-cadherin was negative in all lobular carcinoma cases, making it a preferred antibody choice for the differentiation of infiltrating ductal vs. lobular carcinomas. Combination of rabbit E-cadherin and mouse p120 catenin monoclonal antibody into a multiplex assay will improve the diagnosis of breast cancer types, as well as provide the additional advantages of saving tissue, reagent cost and time.
References
