Value of Claudin-4 Immunostaining in the Diagnosis of Mesothelioma

Nelson G. Ordóñez, MD

Key Words: Claudin-4; Immunohistochemistry; Mesothelioma; Adenocarcinoma

Abstract

Claudin-4 (CL-4) is a tight junction–associated protein that is expressed in most epithelial cells but absent in mesothelial cells. The purpose of this study is to evaluate the utility of CL-4 immunostaining for assisting in the differential diagnosis of mesothelioma. Sixty mesotheliomas (40 epithelioid, 10 biphasic, and 10 sarcomatoid), 185 carcinomas of different origins that can potentially be confused with mesotheliomas, 37 soft-tissue sarcomas, and 5 melanomas were investigated for CL-4 expression. All 60 mesotheliomas were CL-4 negative. In contrast, 169 (91%) of 185 carcinomas expressed this marker. Five of 8 desmoplastic small round cell tumors and the epithelial component of all 5 biphasic synovial sarcomas were CL-4 positive, whereas none of the remaining soft-tissue sarcomas or melanomas expressed this marker. It is concluded that CL-4 is a highly specific and sensitive immunohistochemical marker for assisting in distinguishing epithelioid mesotheliomas from metastatic carcinomas to the serosal membranes.

A well-known characteristic of mesotheliomas is their ability to present a broad range of cytomorphologic features and grow in a variety of histologic patterns. Because of this, mesotheliomas can be difficult to diagnose on routine histologic preparations as they can be confused with a wide array of tumors that can metastasize to the serosal membranes. Because an absolutely specific and sensitive marker for mesotheliomas has not yet been identified, the differential diagnosis of these tumors largely depends on the use of immunohistochemical panels composed of positive mesothelioma markers (ie, those that are frequently expressed in mesotheliomas but not in carcinomas) and positive carcinoma markers (ie, those that are commonly expressed in carcinomas but not in mesotheliomas). The composition of the recommended panels, however, is constantly subject to change as a result of the identification of new markers that can be used in the differential diagnosis of these tumors and the continual publication of new information on the value of the individual markers.

Claudin-4 (CL-4), a transmembrane protein located in the tight junctions (TJs), is widely expressed in most epithelial cells but absent in mesothelial cells. In 2006, Soini et al investigated the potential utility of CL-4 immunostaining in distinguishing epithelioid mesotheliomas from carcinomas metastatic to the serosal membranes. In that study, 7 (29%) of 24 epithelioid, 1 (25%) of 4 sarcomatoid, and none of 7 biphasic mesotheliomas were found to be CL-4 positive, whereas all 23 (100%) metastatic carcinomas expressed this marker. Because the degree of CL-4 positivity in epithelioid mesotheliomas was lower than that observed in adenocarcinomas, the authors concluded that CL-4 immunostaining may have some...
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utility as an additional marker for discriminating between metastatic adenocarcinomas and mesotheliomas. In a subsequent study, Facchetti et al reported expression for this marker in 245 (88%) of 278 primary carcinomas of various sites and 57 (98%) of 58 serosal metastases, whereas all 60 epithelioid, 11 biphasic, and 9 sarcomatoid mesotheliomas included in the study were negative. On the basis of these results, the authors concluded that CL-4 should be considered a primary immunohistochemical marker for assisting in the differential diagnosis of epithelioid mesotheliomas. The purpose of this study is to resolve the discrepancy between the 2 previously mentioned investigations and to discuss the practical utility of CL-4 when compared with other broad-spectrum positive carcinoma markers that are, at present, considered useful for assisting in the differential diagnosis of mesotheliomas.

Materials and Methods

The material used in this study was obtained from the files of the Department of Pathology at the University of Texas M.D. Anderson Cancer Center. Only cases with an unequivocal clinical and pathologic diagnosis of mesothelioma were selected for CL-4 immunostaining. The selection of the nonmesothelial tumors was based on their potential for being confused with the different morphologic subtypes of mesothelioma. Immunohistochemical studies for CL-4 were performed on 5-μm-thick, formalin-fixed, paraffin-embedded tissue sections using the polymeric biotin-free horseradish peroxidase method on a Leica BOND-MAX stainer (Leica Biosystems, Buffalo Grove, IL). The primary antibody used was the 3E2C1 anti–CL-4 mouse monoclonal antibody (Invitrogen, Camarillo, CA; 1:250 dilution). In brief, slides were deparaffinized and hydrated, followed by heat-induced antigen retrieval in which a citrate buffer solution (pH 6.0) was used. Incubation with the primary antibody was followed by development of the immunostaining with 3,3′-diaminobenzidine. The secondary antibody and detection was applied as per instructions from the manufacturer (Leica Biosystems). To evaluate the specificity of the immunoreaction, known positive and negative tissues were used as controls. The immunostaining was graded on a sliding scale of 1+ to 4+ according to the percentage of distinctly reactive cells (1+, 1%-25%; 2+, 26%-50%; 3+, 51%-75%; and 4+, >75%).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Results of Claudin-4 Immunostaining</th>
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<tbody>
<tr>
<td></td>
<td>Total No.</td>
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<tr>
<td>Mesothelioma</td>
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<td>Epithelioid</td>
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<tr>
<td>Biphasic</td>
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<tr>
<td>Sarcomatoid</td>
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<td>Lung</td>
<td></td>
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<tr>
<td>Adenocarcinoma</td>
<td>25</td>
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<tr>
<td>Squamous cell carcinoma</td>
<td>20</td>
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<tr>
<td>Sarcomatoid carcinoma (spindle cell component)</td>
<td>10</td>
</tr>
<tr>
<td>Pleomorphic carcinoma</td>
<td>3</td>
</tr>
<tr>
<td>Small cell carcinoma</td>
<td>3</td>
</tr>
<tr>
<td>Carcinoid tumor</td>
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</tr>
<tr>
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<tr>
<td>Breast ductal carcinoma</td>
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<td>Pancreatic adenocarcinoma</td>
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<td>Colon adenocarcinoma</td>
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<tr>
<td>Prostate adenocarcinoma</td>
<td>2</td>
</tr>
<tr>
<td>Renal cell carcinomas</td>
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<td>Papillary</td>
<td>10</td>
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<tr>
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<tr>
<td>Bladder urothelial carcinoma</td>
<td>2</td>
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<td>Solitary fibrous tumor of the pleura</td>
<td>6</td>
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<tr>
<td>Angiosarcoma</td>
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<td>Malignant fibrous histiocytoma</td>
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<td>Rhabdomyosarcoma</td>
<td>3</td>
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<tr>
<td>Melanoma</td>
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</tbody>
</table>

* Reactivity was defined as follows: 1+, 1%-25%; 2+, 26%-50%; 3+, 51%-75%; and 4+, >75%.
Results

None of the 60 mesotheliomas stained for CL-4 were positive for this marker. Expression was demonstrated, however, in all adenocarcinomas of the lung, pancreas, breast, colon, and prostate and in 44 (98%) of 45 serous carcinomas of the ovary (15 primary, 30 metastatic to the peritoneum) investigated Image 1A and Image 1B. Twenty-eight (85%) of 33 clear cell, 10 (100%) of 10 papillary, and 12 (100%) of 12 chromophobe renal cell carcinomas were also found to be CL-4 positive, as were 19 (95%) of 20 squamous cell and all 3 small cell carcinomas of the lung Image 1C and Image 1D. In the vast majority of these epithelial tumors, the immunoreaction was strong (4+) and occurred along the cell membrane in either a continuous or a punctated staining pattern (Image 1C). Two (67%) of the 3 pleomorphic carcinomas, but only 2 (20%) of the 10 sarcomatoid carcinomas of the lung, were focally positive (1+) for CL-4 in the spindle cell sarcomatoid areas of the tumors, whereas the epithelial areas were consistently positive Image 2A and Image 2B. In all 5 biphasic synovial sarcomas investigated, the positivity for CL-4 was restricted to the epithelial areas of the tumor. The spindle cell component of these tumors and that of all 3 monophasic spindle cell synovial sarcomas were negative for this marker Image 2C. Five (63%) of the 8 desmoplastic

Image 1A, Lung adenocarcinoma showing diffuse strong claudin-4 (CL-4) positivity along the cell membranes (×200). B, Ovarian serous carcinoma displaying membranous positivity for CL-4 (×200). C, Chromophobe renal cell carcinoma demonstrating a punctated staining pattern for CL-4 along the cell membrane (×400). D, Poorly differentiated squamous cell carcinoma of the lung exhibiting strong CL-4 expression (×200).
small round cell tumors investigated were found to express CL-4 Image 2D, whereas all solitary tumors of the pleura, malignant fibrous histiocytomas, angiosarcomas, leiomyosarcomas, rhabdomyosarcomas, and melanomas were negative. The results of the immunostaining are summarized in Table 1.

**Discussion**

Claudins are a large family of transmembrane proteins essential for the formation and maintenance of TJs. TJs or zonula occludens are the most apical component of the junctional complex present on the membranes of epithelial and endothelial cells. TJs visualized by electron microscopy are regions where the outer leaflets of plasma membranes from adjacent cells appear to fuse together and the intercellular space disappears. These structures play a crucial role in the maintenance of cell polarity, cellular arrangement, adhesion, and paracellular transport. To date, 24 human claudins that control the ability of TJs to regulate paracellular transport have been identified. Most tissues express multiple claudins that can interact in both a homotypic and a heterotypic manner to form TJ strands. The exact combination of claudin proteins within a particular tissue is believed to determine the

- **Image 2A**, Pleomorphic lung carcinoma showing strong membranous staining for claudin-4 (CL-4) (×400).
- **Image 2B**, Sarcomatoid carcinoma showing strong reactivity for CL-4 in the better differentiated areas of the tumor (left), while the spindle tumor cells are negative (×200).
- **Image 2C**, Biphasic synovial sarcoma demonstrating strong CL-4 positivity in the epithelial areas of the tumor, while the spindle cell component is negative (×200).
- **Image 2D**, Desmoplastic small round cell tumor exhibiting CL-4 expression (×200).
selectivity and strength of the TJs. CL-4, also known as Clostridium perfringens enterotoxin receptor, is a 209-amino acid protein with a molecular weight of ~22 kDa that is encoded by a gene located on chromosome 7q11.23. CL-4 is widely expressed in most normal epithelial cells, including those of the lung, pancreas, breast, prostate, thyroid, thymus, and bladder.1 It is expressed in collecting ducts and distal convoluted tubules in the kidney, as well as in the biliary duct epithelium in the liver. CL-4 is also expressed in follicular dendritic cells. It is not expressed in hepatocytes or mesothelial cells.1,2,9

Only a few studies have been published on CL-4 expression in mesotheliomas. The first of these investigations was by Soini et al,2 who, using the 3E2C1 anti–CL-4 mouse monoclonal antibody, reported CL-4 expression in 29% of the epithelioid and 25% of the sarcomatoid mesotheliomas investigated. Although the authors indicated that CL-4 reactivity in mesotheliomas was significantly lower than that seen in the metastatic adenocarcinomas to the pleura included in the study, no quantitative grading of the reaction or illustrations of any of the positive mesothelioma cases were provided.

The results of this study are in contrast to those obtained by Facchetti et al,1 who, in a subsequent investigation using the same antibody employed by Soini et al,2 were unable to demonstrate CL-4 expression in any of the 60 epithelioid or 11 sarcomatoid mesotheliomas included in their investigation. That none of the 60 mesotheliomas in the present study were CL-4 positive is an indication that this marker is not expressed in these tumors. The cause of the discrepancy between the results reported by both Facchetti et al and those obtained in the current investigation when compared with those reported by Soini et al is not clear, but it does not appear to be related to the antibody used because the same commercially obtained 3E2C1 anti–CL-4 mouse monoclonal antibody was employed in all 3 studies.

Over the past 3 decades, a large number of immunohistochemical markers that are frequently expressed in carcinomas, but not in mesotheliomas, have been identified. These markers are commonly referred to as positive carcinoma markers and can be subdivided into 2 major groups: broad-spectrum positive carcinoma markers, which are frequently expressed in a wide range of carcinomas, and organ-associated carcinoma markers that, because of their restricted expression, can help to establish the site of origin of a metastatic carcinoma.18 Most of the positive carcinoma markers that have traditionally been used to assist in discriminating between metastatic carcinomas to the serosal membranes and epithelioid mesotheliomas belong to the first group and include MOC-31, Ber-EP4, tumor-associated glycoprotein-72 (TAG-72), carcinoembryonic antigen, BG-8, and CD15, which are the markers that are currently most commonly used in the differential diagnosis between these tumors. Because these markers are usually absent in sarcomatoid carcinomas, they have no utility in distinguishing this type of tumor from sarcomatoid mesotheliomas.

MOC-31 is a monoclonal antibody that reacts with a 40-kDa transmembrane protein known as epithelial cell adhesion molecule (Ep-CAM), which is normally expressed in the basolateral membrane of most epithelial tissues, including simple cuboidal and columnar, pseudostratified columnar, and transitional epithelium.11,12 The vast majority of adenocarcinomas of the lung (86%-100%),13,17 pancreas (100%),18,19 breast (29%-100%),13,14,18 and colon (100%)13,14,18; serous carcinomas of the ovary and peritoneum (97%-100%)20-22; squamous cell carcinomas of the lung (97%-100%)13,23; and urothelial carcinomas (10%-67%)13,18 have been reported to be MOC-31 positive. This is in contrast to epithelioid mesotheliomas, in which 2% to 15% have been reported to be positive in small focal areas or in scattered neoplastic cells.17,21,24,25 Because of its high sensitivity and specificity, MOC-31 is, in my experience, one of the best broad-spectrum positive carcinoma markers for assisting in discriminating between epithelioid pleural mesotheliomas and both lung adenocarcinomas and squamous cell carcinomas of the lung.17,23 As only 50% of the renal cell carcinomas have been reported to react with this antibody, MOC-31 has limited utility in distinguishing these tumors from epithelioid mesotheliomas.26

Ber-EP4 is another mouse monoclonal antibody that, similar to MOC-31, also reacts with Ep-CAM. Published studies have shown that a high percentage of adenocarcinomas of the lung (91%-100%),16,17,27-31 breast (81%-83%),23,32 pancreas (80%-100%),19,32 and colon (100%)19,32; serous carcinomas of the ovary and peritoneum (83%-100%)21,22,29,33,34; squamous cell carcinomas of the lung (74%-100%)23,29,35; and clear cell renal cell carcinomas (27%-42%)26,36,37 react with the Ber-EP4 antibody. In contrast, only 13% to 26% of epithelioid mesotheliomas have been reported to be Ber-EP4 positive, usually in small areas of the tumor or in a few cells.17,21,24,25 The results of these investigations indicate that Ber-EP4 immunostaining can be helpful in assisting in distinguishing epithelioid mesotheliomas from lung adenocarcinomas, serous carcinomas, and squamous cell carcinomas of the lung; however, it has little or no practical utility for assisting in discriminating between epithelioid mesotheliomas and renal cell carcinomas metastatic to the serosal membranes. In my experience, Ber-EP4 is less specific than MOC-31 as I have observed weak positivity for the latter marker in only 2% to 8% of epithelioid mesotheliomas, which is much lower than that seen with Ber-EP413,17,23,26

BG-8 is a mouse monoclonal antibody that recognizes the blood group Lewis x. Published investigations have demonstrated that adenocarcinomas of the lung (58%-100%),17,18,38-41 breast (71%-100%),18,41,42 pancreas (73%),18 prostate (80%-84%),18,41 and colon (76%-90%)18,41; serous

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Am J Clin Pathol 2013;139:611-619
DOI: 10.1309/AJCP0B3YJEXWVJII
carcinomas of the ovary and peritoneum (73%)\(^ {22} \); and squamous cell carcinomas of the lung (80%-87%)\(^ {8,23,40,42} \) are frequently BG-8 positive and that, in most instances, the staining is strong and diffuse. This is in contrast to epithelioid mesotheliomas in which only a minority of cases (3%-9%)\(^ {17,38,41} \) have been reported to exhibit focal reactivity in small areas of the tumor or in sparse cells. Because of these differences in BG-8 reactivity, this marker can be helpful in assisting in distinguishing between epithelioid mesotheliomas and the previously mentioned tumors. BG-8 immunostaining, however, has no utility for assisting in distinguishing between epithelioid mesotheliomas and renal cell carcinomas as only a small percentage (4%-10%)\(^ {18,26} \) of the latter tumors have been reported to be positive for this marker.

TAG-72, also known as B72.3, is one of the earlier broad-spectrum positive carcinoma markers that was found to be useful in assisting in discriminating epithelioid mesotheliomas from metastatic carcinomas to the serosal membranes. Published studies indicate that expression of this marker can be demonstrated in the majority of adenocarcinomas of the lung (70%-100%)\(^ {17,27,30,40,43-50} \), breast (50%-80%)\(^ {42,51-53} \), pancreas (88%-100%)\(^ {44,45} \), and colon (68%-83%)\(^ {49,54} \); serous carcinomas of the ovary and peritoneum (65%-98%)\(^ {20,22,23,36,60} \), and squamous cell carcinomas of the lung (17%-84%)\(^ {40,61,62} \). Epithelioid mesotheliomas usually do not express TAG-72, and only 2% to 11% of these tumors have been reported to be positive in small areas or in sparse cells.\(^ {44,45,50,63,64} \)

CD15 (leu-M1) is another of the earliest broad-spectrum positive carcinoma markers that was found to be useful in assisting in the differential diagnosis between epithelioid mesotheliomas and carcinomas. According to published investigations, CD15 is frequently expressed in adenocarcinomas of the lung (55%-85%)\(^ {15,17,27,28,44,45,48,50,65} \), breast (67%)\(^ {32} \), pancreas (80%)\(^ {57} \), and colon (100%)\(^ {32} \); serous carcinomas of the ovary and peritoneum (30%-80%)\(^ {20,22,23,33,46,57,59,68} \), and squamous cell carcinomas of the lung (13%-45%)\(^ {23,69,70} \). Even though some authors have reported CD15 expression in epithelioid mesotheliomas (2%-29%)\(^ {30,38,44,71,72} \), in my experience, as well as that of other investigators, this marker is not expressed in mesotheliomas.\(^ {17,28,31,45,46,63} \) Because a high percentage of clear cell (62%-71%)\(^ {26,37,73,74} \) and papillary (41%-100%)\(^ {26,37} \) renal cell carcinomas have been reported to express CD15, immunostaining for this marker could assist in distinguishing these tumors from epithelioid mesotheliomas.

Carcinoembryonic antigen (CEA) was the first immunohistochemical marker to become widely accepted as being useful in the differential diagnosis between epithelioid mesotheliomas and lung adenocarcinomas. Current information indicates that CEA is positive in the large majority of adenocarcinomas of the lung (70%-100%)\(^ {16,17,27,28,30,31,45,46,48,50,66,75} \), breast (80%)\(^ {18} \), pancreas (90%-100%)\(^ {18,19,54,76,77} \), and colon (100%)\(^ {18,19,32,54,76} \) and squamous cell carcinomas of the lung (77%-86%)\(^ {18,23} \), whereas epithelioid mesotheliomas are almost invariably negative for this marker.\(^ {17,50,78} \) Therefore, CEA immunostaining can assist in distinguishing epithelioid mesotheliomas from the previously mentioned tumors. In contrast, because CEA is expressed in only a small percentage of serous carcinomas of the ovary and peritoneum (4%-45%)\(^ {20,21,38,58} \), it has no practical utility for discriminating these tumors from mesotheliomas. In addition, because CEA expression is usually absent in renal cell carcinomas, immunostaining for this marker is not useful for distinguishing these tumors from mesotheliomas.\(^ {26} \)

That all adenocarcinomas of the lung, breast, pancreas, colon, and prostate, as well as the vast majority of the serous carcinomas of the ovary, renal cell carcinomas, and squamous cell carcinomas of the lung, in the present study were found to express CL-4, but none of the epithelioid mesotheliomas investigated were positive for this marker, is an indication that CL-4 is a highly specific and sensitive marker that can be used to discriminate between epithelioid mesotheliomas and metastatic carcinomas to the serosal membranes. In addition, when CL-4 is compared with the broad-spectrum positive carcinoma markers that have previously been recommended as being useful in this differential diagnosis, including MOC-31, Ber-EP4, BG-8, TAG-72, CD15, and CEA, both its specificity and/or sensitivity appear, in general, to be higher than all other markers. On the basis of these factors, CL-4 should be considered a primary marker to be included in the panels of immunohistochemical markers used for assisting in the differential diagnosis of epithelioid mesotheliomas. Because only 2 of the 10 sarcomatoid lung carcinomas investigated exhibited staining in small focal areas of the spindle cell component, CL-4 immunostaining does not appear to have significant value in distinguishing these tumors from sarcomatoid mesotheliomas. However, in those tumors that contain small epithelial areas that may not be apparent on routine histology, CL-4 can be helpful in their identification, as shown in Image 2B, and, therefore, may be useful in assisting in the differential diagnosis between these tumors and sarcomatoid mesotheliomas.

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References


