Immunohistochemical Diagnosis of Epithelioid Mesothelioma
An Update

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Objective.—Several new immunohistochemical markers that can assist in the diagnosis of mesotheliomas have been recognized recently. This article reviews the current information available on these markers and also provides a practical approach to the immunohistochemical diagnosis of epithelioid mesotheliomas.

Data Sources.—Current literature concerning immunohistochemical markers for epithelioid mesotheliomas was collected and reviewed.

Study Selection.—Literature emphasizing immunohistochemical diagnosis of epithelioid mesotheliomas was selected.

Data Extraction.—Data deemed helpful to the general surgical pathologist for the diagnosis of epithelioid mesothelioma were included in this review.

Data Synthesis.—Markers identified as potentially useful in the diagnosis of epithelioid mesothelioma include positive markers (namely, calretinin, keratin 5/6, D2-40, podoplanin, mesothelin, and Wilms tumor 1 protein [WT1]) and negative markers (namely, carcinoembryonic antigen, MOC-31, B72.3, and Ber-EP4). Thyroid transcription factor 1 (TTF-1) can assist in determining the lung origin of a carcinoma, and renal cell carcinoma marker (RCC Ma) may help establish its renal origin.

Conclusions.—D2-40 and podoplanin are the 2 most recently recognized markers that have been found to be useful in the diagnosis of epithelioid mesotheliomas. Since D2-40 and podoplanin appear to be highly sensitive and specific for epithelioid mesotheliomas, either may be considered for inclusion in the battery of antibodies currently recommended for distinguishing epithelioid mesotheliomas from metastatic carcinomas. However, it should be kept in mind that their utility has not yet been fully determined in routine diagnostic work.

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The distinction of epithelioid mesotheliomas from adenocarcinomas metastatic to the serosal membranes is one of the most challenging diagnostic problems faced by surgical pathologists. Because epithelioid mesotheliomas can exhibit many histologic patterns, they may be confused with a variety of carcinomas. Epithelioid pleural mesotheliomas must be distinguished from peripheral adenocarcinomas of the lung involving the pleura and from metastatic adenocarcinomas arising in a distant organ (eg, kidney). In the peritoneum, malignant mesotheliomas may resemble papillary peritoneal serous carcinomas or metastatic serous carcinomas of the ovary. Of the various ancillary techniques that have been recognized as being useful in the differential diagnosis of epithelioid mesotheliomas, immunohistochemistry is considered to have the most practical utility.

Since the first report by Wang et al1 more than 25 years ago indicating that carcinoembryonic antigen (CEA) could serve as an immunohistochemical marker in the diagnosis of mesothelioma, a large number of other markers that are helpful in this diagnosis have been investigated. At present, however, an absolutely specific marker for mesothelioma has not yet been recognized. Because of the lack of a specific marker for mesothelioma, the immunohistochemical diagnosis of this tumor largely depends on the use of panels of markers that combine those that are frequently expressed in mesotheliomas (positive mesothelioma markers) with those that are most commonly present in carcinomas (negative mesothelioma markers). These panels, however, are continually changing as a result of the recognition of new markers that can assist in the diagnosis of these tumors. The purpose of this article is to discuss the value of these new markers and to determine the current utility of those that at one time were believed to have some application in establishing the differential diagnosis between epithelioid mesotheliomas and adenocarcinomas. Additionally, because expression of these markers varies among the different types of carcinomas, I review the panels of markers for which there is evidence indicating utility in distinguishing these tumors from epithelioid mesotheliomas.

POSITIVE MARKERS

Monoclonal Antibody D2-40

D2-40 is a recently developed, commercially available monoclonal antibody that reacts with a 40-kd antigen (bet-
ter known as the oncofetal M2A antigen) in fetal germ cells and germ cell tumors.² Because the antigen recognized by this antibody is selectively expressed in lymphatic endothelium, it has been shown that it could be very helpful in both the diagnosis of lymphatic derived tumors and in determining lymphatic invasion by tumors.³⁴ Recent investigations have also shown that because the antibody often reacts with epithelioid mesotheliomas, but not with carcinomas, it could be useful in discriminating between these malignancies.¹⁻⁶ A 2005 study by Chu et al⁵ investigated the value of D2-40 as a mesothelioma marker. In that study, strong membranous reactivity was reported in 33 (100%) of 33 epithelioid mesotheliomas and in the epithelial component of 15 (94%) of 16 biphasic mesotheliomas.⁵ Additionally, focal, weak, membranous positivity was observed in 17 (65%) of 26 serous carcinomas, but no membranous staining was seen in any of the other carcinomas investigated. In 2 separate investigations using different cohorts of mesothelioma cases, I found membranous positivity for D2-40 in 25 (86%) of 29 and in 37 (93%) of 40 epithelioid mesotheliomas.⁷⁸ In the large majority of cases, the reaction was diffuse and occurred primarily along the apical surface of the cells (Figure, A). Among the different types of carcinomas investigated, only about 15% of the serous carcinomas presented membranous positivity, but in contrast to the epithelioid mesotheliomas, the reaction tended to be focal. None of the other carcinomas were positive for D2-40. These findings indicate that D2-40 is one of the most sensitive and specific markers for the diagnosis of epithelioid mesothelioma.

**Podoplanin**

Podoplanin is another recently recognized mesothelioma marker. It is an approximately 38-kd membrane mucoprotein that was originally detected on the surface of rat glomerular epithelial cells (podocytes) and was found to be linked to the flattening of the foot processes in puromycin-induced nephrosis.⁹ Like D2-40, podoplanin has been found to be expressed in lymphangiomas, Kaposi sarcomas, and in a subgroup of angiosarcomas that are presumably of lymphatic origin. Podoplanin is not expressed in the endothelial cells of blood vessels.¹⁰ Recently, Kimura and Kimura¹¹ reported podoplanin expression in 5 of 5 epithelioid mesotheliomas, but in none of 93 adenocarcinomas of various origins, and suggested that this marker could assist in distinguishing between these malignancies. In 2 comparative studies, I found that podoplanin exhibited sensitivity and specificity values similar to those of D2-40 in the diagnosis of mesothelioma (Figure, B).⁷⁸

**Mesothelin**

Mesothelin is a 40-kd differentiation antigen that was first described as the antigenic target of the K1 monoclonal antibody, which was generated using the OVCAR-3 ovarian cell line as immunogen. In 1992, using the K1 antibody on frozen tissue specimens, Chang et al¹² were able to demonstrate mesothelin expression in all 15 epithelioid mesotheliomas tested, but in none of the 23 lung adenocarcinomas they investigated. The authors concluded that immunostaining for this marker could be useful in the differential diagnosis of these malignancies. Despite these promising results, no further investigations on the potential utility of this marker in the diagnosis of mesothelioma were published until the 5B2 anti-mesothelin antibody became commercially available. In 2003, using the 5B2 antibody on formalin-fixed, paraffin-embedded specimens, I reported strong mesothelin expression in all 44 (100%) epithelioid mesotheliomas,¹³ thus confirming the observations of Chang et al¹² that mesothelin is a highly sensitive marker for epithelioid mesothelioma. However, in contrast to Chang et al, I was able to demonstrate mesothelin positivity in 12 (39%) of 31 lung adenocarcinomas. In the lung adenocarcinomas, the staining was focal and often cytoplasmic, while in the epithelioid mesotheliomas, it was usually strong and diffuse, and occurred along the cell membrane (Figure, C). Aside from the lung adenocarcinomas, mesothelin was also expressed in a variety of other carcinomas, especially in nonmucinous carcinomas of the ovary and ductal adenocarcinomas of the pancreas, in which the large majority of these tumors expressed this marker. Despite its low specificity for mesothelioma and because of the common and strong membranous mesothelin expression in epithelioid mesotheliomas, negative staining for this marker is a strong indication against such a diagnosis. Because mesothelin is commonly expressed in mesotheliomas, recent investigations¹⁴ have indicated that this protein could serve as a serum marker for monitoring disease progression in mesothelioma patients and for screening asbestos-exposed individuals for early evidence of this disease. Additionally, mesothelin is now being considered as a potential target for immunotherapy.¹⁵

**Calretinin**

The first reports indicating that calretinin was a useful immunohistochemical marker for the diagnosis of mesothelioma were published independently in 1996 by Doglioni et al¹⁶ and Gotzos et al.¹⁷ Following these publications, other articles appeared in the literature that confirmed these initial reports¹⁶,¹⁷; however, other investigators found that this marker had no utility for the diagnosis of mesothelioma.¹⁸,¹⁹ In 1998 in a comparative immunohistochemical investigation using a polyclonal antibody raised in rabbits against human recombinant calretinin and another against guinea pig calretinin, it was determined that the cause of the conflicting results was the type of antibody used in the different studies.²² Only antibodies against human recombinant calretinin have proven to be useful in the diagnosis of mesothelioma.²³,²² At present, calretinin is regarded as being the most sensitive and one of the most specific of the positive mesothelioma markers. In contrast to other highly sensitive mesothelioma markers, such as mesothelin, D2-40, podoplanin, and keratin 5/6, which are commonly expressed in epithelioid mesotheliomas, but not in sarcomatoid mesothelioma, calretinin is frequently expressed in all histologic types of mesothelioma. Using polyclonal antibodies against human recombinant calretinin, different groups of investigators have been able to demonstrate calretinin expression in all epithelioid mesotheliomas investigated; therefore, negative staining for this marker should be considered an indication against such a diagnosis.¹⁸ The percentage of adenocarcinomas that have been reported to show calretinin expression with polyclonal antibodies to human recombinant calretinin has ranged from 0% to 38% of the cases.³¹

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* References 16, 18, 19, 22, 25, 28–31.
† References 16, 18, 19, 22, 23, 25, 26, 28, 29, 32.
The calretinin staining pattern in epithelioid mesothelioma is typically strong and diffuse, and occurs in both the nucleus and the cytoplasm (Figure, D). In contrast, the reactivity in adenocarcinomas is often confined to small focal areas of the tumor. On rare occasions, however, diffuse reactivity can occur in adenocarcinomas. Additionally, differences in calretinin expression exist among different types of carcinomas. In recent investigations, the percentage of calretinin positivity ranged from 6% to 10% in lung adenocarcinomas,24-26,28,29 31% to 38% in serous carcinomas,5,8,32 and 0% to 4% in renal cell carcinomas.30,33,34 Squamous carcinomas of the lung also express this marker in 23% to 39% of the cases.22,33,35

Recently, a monoclonal antibody to human recombinant calretinin (calret-1) became commercially available. In a study published in 2005, Granville et al.36 compared the specificity and sensitivity of this antibody for the diagnosis of mesothelioma with a commercial polyclonal an-
tibody to human recombinant calretinin. Based on the results obtained, these investigators concluded that both antibodies had a similar specificity for mesothelioma, but the sensitivity of the polyclonal antibody was slightly higher than that of the monoclonal.

Wilms Tumor 1 Protein

Wilms tumor 1 (WT1) protein is one of the most recent markers introduced for the diagnosis of mesothelioma. The first studies using commercially obtained polyclonal antibodies were published in 2000. The percentage of WT1 positivity reported in mesotheliomas using these antibodies has ranged from 43% to 75%. Higher percentages of WT1 expression, however, have been reported using the commercially available 6F-H2 anti-WT1 monoclonal antibody. In a recent study using this antibody, 56 (93%) of 60 epithelioid mesotheliomas, but none of 50 lung adenocarcinomas, were found to express WT1. Because of its high sensitivity and absolute specificity, WT1 is one of the best positive markers for discriminating between these malignancies. However, since WT1 is commonly expressed in serous carcinomas, immunostaining for this marker has no utility in distinguishing between epithelioid mesotheliomas and serous carcinomas. Only a few studies have investigated WT1 expression in renal cell carcinomas. In one of these investigations, WT1 expression was reported in only 1 of 24 conventional renal cell carcinomas, but in none of the papillary, chromophobe, or sarcomatoid renal cell carcinomas investigated.

Keratins 5 and 6

The possibility that keratins 5 and 6 could serve as markers for distinguishing mesotheliomas from lung adenocarcinomas was first suggested by Blobel et al. in the mid-1980s. This observation was not confirmed until relatively recently, however, when the D5/16B4 monoclonal antibody, which is highly specific for these keratins, became commercially available. Using this antibody, the percentage of keratin 5/6 expression reported in the literature has ranged from 64% to 100% in epithelioid mesotheliomas, 0% to 19% in lung adenocarcinomas, and 22% to 35% in serous carcinomas. In my experience, nearly all epithelioid mesotheliomas express keratin 5/6; however, because the staining can be focal, it is important to be aware that a false-negative result may be obtained when the immunostaining is performed on a small biopsy specimen. Positivity for keratin 5/6 in lung adenocarcinomas is uncommon, and when it occurs, it is usually in small focal areas of the tumor or in individual cells. This finding has been interpreted as probably being due to the presence of squamous differentiation, which is known to occur in pulmonary adenocarcinomas. Because about one third of serous carcinomas express keratin 5/6, immunostaining for this marker has no practical utility for distinguishing these tumors from mesotheliomas. However, since it is not expressed in renal cell carcinomas, keratin 5/6 can assist in distinguishing epithelioid mesotheliomas from these tumors. Squamous carcinomas of the lung usually express keratin 5/6, and in most instances, the staining is strong and diffuse; therefore, this marker has no utility in distinguishing these tumors from mesotheliomas. In addition to D5/16B4, other monoclonal antibodies that react with either keratin 5 or 6 on routinely fixed and processed specimens have become commercially available.

In their 2003 study using the XM26 monoclonal antibody, which reacts exclusively with keratin 5, Miettinen and Sarlomo-Rikala investigated the utility of this antibody in discriminating between epithelioid mesotheliomas and various types of lung carcinomas. Twenty-six (93%) of 28 mesotheliomas were positive with this marker, while only 25 (9.8%) of 254 pulmonary adenocarcinomas exhibited focal positivity. These results are comparable to those reported by others using the D5/16B4 anti-keratin 5/6 monoclonal antibody.

Thrombomodulin

Thrombomodulin (CD141) was the first of the positive mesothelioma markers that proved useful in the diagnosis of this tumor. In recent years, however, the importance of this marker has declined, owing to the recognition of more sensitive and specific mesothelioma markers. The reported percentage of thrombomodulin-positive epithelioid mesotheliomas has ranged from 34% to 100% of cases, and for lung adenocarcinomas from 5% to 77%. In a recent investigation, I found that 46 (77%) of 60 epithelioid mesotheliomas and just 7 (14%) of 50 lung adenocarcinomas expressed this marker. In the latter tumors, the reactivity was limited to small focal areas or scattered neoplastic cells, while in the mesotheliomas, it was stronger and tended to be diffuse. Thrombomodulin expression is typically manifested by a membranous staining pattern. Areas of tumor necrosis or degeneration often exhibit strong cytoplasmic staining, which is probably the result of passive adsorption of the antigen from the serum, not true thrombomodulin expression. In addition to epithelioid mesotheliomas, angiosarcomas and squamous carcinomas often express thrombomodulin. This pattern is important to keep in mind, because these tumors can potentially be confused with epithelioid mesotheliomas.

NEGATIVE MARKERS

Monoclonal Antibody MOC-31

MOC-31 is a monoclonal antibody that recognizes the epithelial cell adhesion molecule (Ep-CAM), also known as human pancarcinoma-associated epithelial glycoprotein-2 (EGP-2). Because this antibody reacts with most carcinomas, but rarely and only patchily with epithelioid mesotheliomas, at present it is considered to be one of the most sensitive and specific among the so-called negative mesothelioma markers for discriminating between epithelioid mesotheliomas and adenocarcinomas. Nearly all lung adenocarcinomas and serous carcinomas of the ovary strongly react with this antibody, and the staining pattern is usually strong and diffuse (Figure, E). In contrast, positive reactions in small focal areas or in scattered cells have been reported in only 5% to 10% of epithelioid mesotheliomas. Approximately 50% of renal cell carcinomas stain with the MOC-31 antibody; thus, this marker has limited utility for discriminating these tumors from mesotheliomas.

CD15 (Leu-M1)

CD15 (Leu-M1) was one of the first markers to become widely used in the diagnosis of mesothelioma. Although some studies have reported CD15 in as many as 32% of mesotheliomas, in my experience as well as that of most investigators, this marker is not expressed in these tumors. Similarly, a wide range has been reported in the percentage of CD15 expression in adenocarcinomas,
varying from 28% up to 100% of the cases. According to current information, about 70% to 75% of lung adenocarcinomas and 30% to 60% of serous carcinomas of the ovary and peritoneum express CD15. Even though CD15 is a highly specific marker for discriminating between epithelioid pleural mesotheliomas and lung adenocarcinomas, its sensitivity is relatively low when compared with other negative mesothelioma markers that are currently available. It should be mentioned, however, that because the large majority of conventional and papillary renal cell carcinomas have been found to express CD15, immunostaining for this marker can be very useful in distinguishing epithelioid mesotheliomas from renal cell carcinomas.

Monoclonal Antibody BG-8

BG-8 is a monoclonal antibody that recognizes the blood group antigen Lewis. In 1997, Riera et al. used this antibody to demonstrate Lewis expression in 187 (89%) of 211 adenocarcinomas of various origins. Because the majority of adenocarcinomas exhibited diffuse strong positivity, while staining in mesotheliomas was focal and weak, these investigators concluded that BG-8 immunostaining could assist in differentiating epithelioid mesotheliomas from adenocarcinomas involving the serosal membranes. In 2 recent investigations, I was able to demonstrate Lewis expression in 96% of lung adenocarcinomas and in 73% of serous carcinomas of the ovary, in most of these cases, the staining was strong and diffuse. In contrast, only 3% to 7% of the epithelioid mesotheliomas exhibited focal positivity in small areas or in sparse cells. The conclusion of both investigations was that BG-8 could be useful in differentiating epithelioid mesotheliomas from both lung adenocarcinomas and serous carcinomas. Because renal cell carcinomas often do not react with this antibody, it has no utility for distinguishing these tumors from mesotheliomas.

Ber-EP4 Monoclonal Antibody

In 1990, Latza et al. published the first study indicating that immunostaining with the Ber-EP4 antibody could assist in the diagnosis of mesotheliomas; these investigators reported reactivity in 142 (99%) of 144 carcinomas of various origins, but in none of 14 mesotheliomas. In a similar study published 2 years later, Gaffey et al. reported Ber-EP4 positivity in 103 (86%) of 120 adenocarcinomas of various origins, as well as in 10 (20%) of 49 mesotheliomas. These investigators concluded that Ber-EP4 positivity did not exclude a diagnosis of mesothelioma, even though the staining seen in adenocarcinomas was usually strong and diffuse, while that in mesotheliomas was always focal. Since then, many other studies have been published confirming that mesotheliomas can present Ber-EP4 reactivity, although disagreements still exist regarding the percentage of mesotheliomas that can be positive for this marker, which according to some of the most recent studies, can be as many as one third of the cases. Additionally, a wide range of reactivity for this marker has also been reported in adenocarcinomas, ranging from as low as 32% in some studies to 100% in others. The disparity in the results reported in the different series can be attributed to a variety of factors, such as the site of origin of the adenocarcinoma. For example, although pulmonary adenocarcinomas and serous carcinomas almost invariably stain with the Ber-EP4 antibody, only 35% to 50% of renal cell carcinomas do. Another factor that may have affected the results is the interpretation of the immunohistochemical reaction. In some investigations a single cell reaction was considered to be an indicator of tumor positivity, a cutoff level was used in others. Additionally, some investigators have indicated that only tumors that present a lateral membranous staining pattern should be considered as truly positive. Because both lung adenocarcinomas and serous carcinomas often stain strongly with the Ber-EP4 antibody, strong and diffuse reactivity for this marker can be useful in discriminating these tumors from both epithelioid pleural mesotheliomas and peritoneal mesotheliomas (Figure F). However, when the staining is focal, the value of this marker is very limited.

Carcinoembryonic Antigen

Carcinoembryonic antigen was the first marker to be generally accepted as useful in distinguishing between epithelioid mesothelioma and pulmonary adenocarcinoma. Current information indicates that about 80% of lung adenocarcinomas express CEA, while mesotheliomas are almost invariably negative for this marker. Because of its high specificity and sensitivity, CEA is one of the best negative mesothelioma markers available to assist in the differential diagnosis of epithelioid mesotheliomas and adenocarcinomas of the lung. It should be emphasized, however, that the value of CEA immunostaining in distinguishing between epithelioid mesotheliomas and metastatic nonpulmonary adenocarcinomas largely depends on the site of origin of the adenocarcinoma. For example, even though adenocarcinomas of the gastrointestinal tract, especially those originating in the colon, are usually positive for CEA, reactivity is absent in renal cell carcinomas, and only a minority of the serous carcinomas of the ovary and peritoneum express this marker. In a comparative investigation, only 7 (16%) of 45 serous carcinomas of the ovary and peritoneum were positive for CEA, indicating that while CEA may be a useful immunohistochemical marker in discriminating between epithelioid pleural mesotheliomas and lung adenocarcinomas, it has little value in separating epithelioid peritoneal mesotheliomas from primary or metastatic serous carcinomas involving the peritoneum. Because CEA is not expressed in renal cell carcinomas, it has no utility in distinguishing epithelioid mesotheliomas from renal cell carcinomas metastatic to the serosal membranes.

Monoclonal Antibody B72.3

B72.3 is a monoclonal antibody that reacts with a tumor-associated protein, TAG-72. Because it is often expressed in a wide variety of adenocarcinomas, but not in epithelioid mesotheliomas, B72.3 is commonly used in discriminating between these malignancies. Despite general agreement that B72.3 immunostaining is helpful in this differential diagnosis, differences of opinion exist regarding the percentage of adenocarcinomas (range, 35%–100%) and epithelioid mesotheliomas (range, 0%–48%) that react with this antibody. One of the largest studies on the value of B72.3 was published by Riera et al. in 1997; these investigators reported positivity in 2 (3.5%) of 57 epithelioid mesotheliomas and in 170 (80.5%) of 211 adenocarcinomas of various origins. I investigated the value of B72.3 in several studies, the largest of which was published in 1997. In that analysis, the percentage of adenocarcinomas (range, 35%–100%) and epithelioid mesotheliomas (range, 0%–48%) that react with this antibody.
mas and 89 (81%) of 110 pulmonary adenocarcinomas reacted with this antibody.

In a study published the following year, the value of B72.3 immunostaining in distinguishing peritoneal epithelioid mesotheliomas from papillary serous carcinomas was investigated. Thirty-nine (87%) of 45 serous carcinomas involving the ovary and peritoneum, but none of the mesotheliomas tested were positive for B72.3. Two recent investigations reported similar results. Despite the availability of a large number of new markers, B72.3 remains one of the best markers for distinguishing between epithelioid mesotheliomas and both lung adenocarcinomas and serous carcinomas involving the ovary and peritoneum. Because B72.3 does not react with renal carcinomas, however, it has no utility for distinguishing these tumors from mesotheliomas.

Monoclonal Antibody CA 19-9

CA 19-9 is a sialylated lacto-N-fucopentose II related to the Lewis blood group. It is commonly expressed in tumors of the gastrointestinal tract, pancreas, and ovary. Because of its frequent expression in ovarian carcinomas, but not in mesotheliomas, several studies have investigated the utility of CA 19-9 in distinguishing primary and metastatic serous carcinomas involving the peritoneum from epithelioid mesotheliomas. In a recent investigation, I found that CA 19-9 expression was demonstrated in 67% of serous carcinomas, but in none of the mesotheliomas tested. This finding indicates that even though this marker is 100% specific for discriminating between serous carcinomas and peritoneal mesotheliomas, its sensitivity is rather low, thus limiting its practical utility. Some studies have also investigated the value of CA 19-9 in separating lung adenocarcinomas from pleural mesotheliomas. Because only 39% to 53% of lung adenocarcinomas have been found to be positive for CA 19-9, this marker is not useful in distinguishing these tumors from pleural mesotheliomas.

Miscellaneous Markers

E-Cadherin and N-Cadherin

In 1995, Peralta-Soler et al were the first to investigate the potential utility of E-cadherin and N-cadherin for discriminating between epithelioid mesotheliomas and lung adenocarcinomas. In that study, strong N-cadherin staining was reported in all of the mesotheliomas tested, whereas only a few adenocarcinomas showed positivity in a limited number of cells. Conversely, all adenocarcinomas of the lung were strongly positive for E-cadherin, while only a minority of the mesotheliomas exhibited positivity in a few cells. The conclusion of this study was that, based on the differences in their expression, immunostaining for these 2 markers could assist in discriminating between epithelioid mesotheliomas and pulmonary adenocarcinomas. Following this publication, many other studies appeared in the literature, and while some confirmed Peralta-Soler et al’s observations, others found that neither E-cadherin nor N-cadherin had any utility in this differential diagnosis. I recently investigated the expression of these markers using several monoclonal antibodies to E-cadherin and N-cadherin in an attempt to resolve this controversy. Based on the results of my investigation, it appears likely that the disparate results reported in the literature on the value of these markers can be attributed, at least in part, to differences in the reactivity of the various antibodies used in the different studies. I also concluded that neither E-cadherin nor N-cadherin had any practical utility in the diagnosis of mesotheliomas.

Lung-Associated Markers

Thyroid Transcription Factor 1.—Thyroid transcription factor 1 (TTF-1) is a nuclear tissue–specific protein transcription factor that is expressed in normal lung and thyroid, as well as in the tumors derived from these organs. At present, there are several highly specific commercial monoclonal antibodies to TTF-1 that can be used on formalin-fixed, routinely processed specimens. Most of the studies published on TTF-1 expression in tumors have used the 8G7G3/1 monoclonal antibody. The reported percentage of TTF-1 positivity has ranged from 58% to 97% in lung adenocarcinomas, while none of the mesotheliomas investigated have been positive for this marker. Squamous carcinomas usually do not express TTF-1.

Surfactant Apoproteins.—Most of the information on the value of surfactant apoproteins as tumor markers has been obtained using the recently available PE-10 monoclonal antibody, which reacts exclusively with surfactant apoprotein A. The percentage of positivity reported with this antibody has ranged from 42% to 73% in lung adenocarcinomas. Because of its relatively low sensitivity for lung adenocarcinomas, immunostaining for surfactant apoprotein A has, in my experience, little practical utility in the differential diagnosis of epithelioid mesotheliomas and lung adenocarcinomas, especially when compared with TTF-1.

Kidney-Associated Markers

Renal Cell Carcinoma Marker.—Renal cell carcinoma marker (RCC Ma) is the name given to a recently commercially available monoclonal antibody that can be used successfully on formalin-fixed, routinely processed specimens. This antibody was generated using the microsomal fraction of human renal cortical tissue as immunogen and reacts with a 200-kd glycoprotein present in the normal proximal tubule epithelial cells. In 2001, McGregor et al reported RCC Ma positivity in 122 (80%) of 153 primary renal cell carcinomas (84% of the clear cell, 96% of the papillary, 45% of the chromophobe, 25% of the sarcomatoid, and none of the collecting duct were positive). Forty-two (67%) of 63 metastatic renal cell carcinomas were also positive. In contrast, only a few non–renal cell carcinomas, most of which originated in the breast, exhibited RCC Ma positivity. In a subsequent comparative study published in 2004, the value of RCC Ma for discriminating between renal cell carcinomas and mesotheliomas was investigated. In that study, 75% of the conventional and papillary carcinomas, but none of the chromophobe or sarcomatoid carcinomas, were RCC Ma positive. Only 3 (8%) of the 40 epithelioid mesotheliomas exhibited positivity for this marker, which was limited to a few cells or small focal areas of the tumor.

CD10.—CD10, also known as common acute lymphoblastic leukemia antigen (CALLA), is a cell surface–neutral endopeptidase that inactivates bioactive peptides. Because CD10 is frequently expressed in renal cell carcinomas, some investigators have indicated that immunostaining for this marker can assist in distinguishing these tumors from other malignancies with which they may be confused. In a recent comparative study, the potential...
utility of this marker for discriminating between renal cell carcinomas and mesotheliomas was investigated. Because the large majority of renal carcinomas and 48% of epithelioid mesotheliomas were found to express CD10, it was concluded that this marker had no utility in discriminating between these malignancies.

**Keratins 7 and 20**

Several investigations have demonstrated that the combined use of keratin 7 and keratin 20 immunostaining can assist in establishing the site of origin of a metastatic carcinoma in a patient with no known primary tumor. Because epithelioid mesotheliomas, lung adenocarcinomas, and serous carcinomas of the ovary and peritoneum almost invariably present strong reactivity for keratin 7 and are negative for keratin 20, immunostaining for these 2 keratin peptides has no utility in the differential diagnosis of these malignancies. However, negative staining for both keratin 7 and keratin 20, or positive staining for keratin 20 is a strong indication that the tumor is not a mesothelioma, lung adenocarcinoma, or serous carcinoma. It should be mentioned, however, that on rare occasions mesotheliomas can present focal positivity for keratin 20, but these cases are always associated with strong keratin 7 expression.

**CONCLUSIONS AND RECOMMENDATIONS**

D2-40 and podoplanin are the 2 markers recognized most recently as being useful in the diagnosis of epithelioid mesotheliomas. They are expressed in approximately 90% of epithelioid mesotheliomas, but with the exception of serous carcinomas, they do not appear to be expressed in any other type of carcinoma. Because D2-40 and podoplanin appear to be highly sensitive and specific for epithelioid mesotheliomas, either of these markers may be considered for inclusion in the battery of antibodies currently recommended for distinguishing epithelioid mesotheliomas from carcinomas metastatic to the serosal membranes. However, it should be kept in mind that their utility has not yet been fully determined in routine diagnostic work. It appears that the use of one of these markers in combination with one of the positive (calretinin, keratin 5/6, or WT1) and one of the negative (CEA, MOC-31, Ber-EP4) mesothelioma markers would allow mesotheliomas to be distinguished from adenocarcinomas. WT1, however, is commonly expressed in serous carcinomas; therefore, this marker has no utility in distinguishing these tumors from peritoneal mesotheliomas. In addition, because CEA is rarely expressed in serous carcinomas and is absent in renal cell carcinomas, this marker has no utility in distinguishing either of these tumors from epithelioid mesotheliomas. Among the tissue-associated markers, TTF-1 can assist in determining the lung origin of a carcinoma, and RCC Ma may help establish its renal origin.

**References**