Podoplanin Is a Useful Marker for Identifying Mesothelioma in Malignant Effusions

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The diagnosis of malignant mesothelioma in serosal effusions continues to be a major challenge because some of its cytomorphological features closely resemble adenocarcinomas. Immunohistochemistry is a valuable tool in the differentiation of epithelioid mesothelioma from metastatic adenocarcinomas. However, no single antibody has demonstrated absolute sensitivity or specificity. In this study, we evaluated the value of immunostaining pattern for podoplanin to differentiate mesothelioma from adenocarcinomas of various origins.

Cell blocks from previously collected paraffin-embedded cell blocks of 86 effusions (18 mesothelioma, 35 reactive mesothelium, 9 breast adenocarcinoma, 14 ovarian adenocarcinoma, and 10 lung adenocarcinoma) were retrieved from the file of the Department of Pathology at University of Michigan and Lund University in Sweden and were used for the study. Slides prepared from the cell blocks were stained for podoplanin. The percentage of immunostained cells was recorded as follows: 1+ (5–25%), 2+ (26–50%), and 3+ (>50%). A stain result involving <5% of cells was considered negative. The intensity of positive results was evaluated as strong, moderate, or weak.

Podoplanin is expressed in 94% of malignant mesothelioma cases (17/18), 97% (30/31) of cases of reactive mesothelial, 0% of lung adenocarcinoma cases (0/9), 0% of breast adenocarcinoma (0/9), and 7% of ovarian adenocarcinoma (1/14). All positive cases of malignant mesothelioma and reactive mesothelium showed strong membranous reactivity to podoplanin. The one positive case of ovarian adenocarcinoma showed a weak membranous podoplanin immunostaining.

On the basis of our results and published data, we believe that membranous podoplanin immunoreactivity, in conjunction with calretinin, would be more specific than CK5/6 and WT-1 in differentiating epithelioid malignant mesothelioma from adenocarcinoma of the lung, breast, and ovary. Diagn. Cytopathol. 2010;38:264–269. © 2010 Wiley-Liss, Inc.

Key Words: podoplanin; mesothelioma; malignant effusion

Malignant pleural mesothelioma arises from the serosal surfaces of body cavities and is linked to asbestos exposure. It is relatively rare in frequency but with poor clinical outcomes mainly because of lack of effective treatment at present.[1,2] Therefore, accurate diagnosis of malignant mesothelioma and correctly differentiating it from other tumors is imperative for proper patient management.

However, the diagnosis of malignant mesothelioma continues to be a major challenge because of its ability to exhibit a broad range of cytomorphological features and to grow in a wide variety of histologic patterns. The tumor cells can exhibit epithelial, sarcomatous, and biphasic differentiation.[3] Epithelial malignant mesothelioma is composed of epithelial cells arranged in tubules, papillary patterns, and many other histologic patterns that closely resemble adenocarcinomas.[4]

The diagnosis of epithelioid mesothelioma in the cytological specimens has been greatly facilitated by the use of immunohistochemistry in the cell blocks. There are several lines of evidence indicating that immunohistochemistry is a valuable tool in the differentiation of epithelioid mesothelioma from metastatic adenocarcinomas.[5] However, no single antibody has demonstrated absolute sensitivity or specificity. Therefore, it is a common practice to use a panel of markers that combine those that are frequently expressed in mesothelioma with those that are commonly expressed in carcinomas.

It has been shown that the antibody D2-40, originally raised against M2A protein expressed in germ cell tumors, recognizes podoplanin.[6] Podoplanin and D2-40 have recently been recognized to stain mesothelial cells.[7–9] Both markers are known to recognize lymphatic endothelium with high sensitivity and specificity.[10] Podoplanin is a sialoglycoprotein that was first recognized as the E11

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antigen, expressed by rat osteoblasts and osteocytes.[11] Later, it was named as podoplanin because it was found on the surface of rat glomerular podocytes and was linked to the effacement of foot processes in glomerular disease.[12] Currently, it is most widely used as a selective marker for lymphatic endothelium. It has also been identified in other normal tissues including mesothelium, myoepithelial cells, follicular dendritic cells, and basal keratinocytes, as well as neoplasms including angiosarcomas, germ cell tumors, and squamous cell carcinomas.[13–16]

The precise functions of podoplanin are not very clear. However, podoplanin has been associated with increased tumor cell motility and invasion.[17] Podoplanin expression shows membranous localization in epithelioid mesothelioma and cytoplasmic localization in sarcomatoid mesothelioma.[18]

The purpose of this study is to evaluate the prospective usefulness of podoplanin in the workup of effusions.

Materials and Methods

Cell blocks from previously collected paraffin-embedded blocks of pleural effusions were retrieved from the file of the Department of Pathology at University of Michigan, Ann Arbor, MI and Lund University in Sweden. The study included a total of 86 cases. These were comprised of 18 cases of epithelioid mesothelioma, 35 cases of reactive mesothelium, 9 cases of breast ductal adenocarcinoma, 14 cases of ovarian serous adenocarcinoma, and 10 cases of lung adenocarcinoma. Immunostaining for podoplanin was performed on paraffin sections as recommended by the manufactures. Mouse monoclonal antibody against podoplanin was obtained from Vector Laboratory (Burlingame, CA). Antibody against podoplanin was used at 1:200 dilution. Briefly, staining procedure was conducted using an automated immunostainer on 5-μm thick sections of paraffin-embedded tissue. Sections were deparaffinized in xylene and rehydrated in a descending ethanol series. The antigens were retrieved using citrate buffer. Endogenous peroxidase activity was blocked by immersion for 10 minutes in 0.3% hydrogen peroxide in methanol solution, followed by a single wash in phosphate buffered saline (pH 7.4). The immunostaining was developed using 3,3′-diaminobenzidine as chromogen. Appropriate positive and negative control tissues were added on each automated immunohistochemistry run to confirm antibody specificity. Immunoreactivity was scored as negative (no immunostaining) or positive. Positive results were evaluated as strong, moderate, or weak. The percentage of immunostained cells was recorded as follows: 1+ (5–25%), 2+ (26–50%), and 3+ (>50%). A stain result involving <5% of cells was considered negative. The intensity of staining was assessed as strong, moderate, or weak using the podoplanin staining intensity in malignant mesothelioma cells as a reference for strong intensity.

Table I. Immunohistochemistry Staining for Podoplanin Results (Membranous Pattern)

Tissue type	*Total number (n)*	*Number positive*	*Percent positive*
Reactive mesothelia	31	30	97
Mesothelioma	18	17	94
Adenocarcinoma			
Lung	9	0	0
Breast	9	0	0
Ovary	14	1	7
Total adenocarcinoma	32	1	3

Results

A total of 86 cases were used in this study. Four cases of reactive mesothelium and one case of lung adenocarcinoma were excluded from the study because of very low cellularity. The immunohistochemical results are summarized in Tables I and II.

Podoplanin was found to be expressed in 17/18 cases of malignant mesothelioma (94%) with more than 50% of cells expressing a membranous pattern. The intensity of membranous staining was strong in eight cases (44%), moderate in five cases (28%), and weak in four cases (22%). Another case (6%) showed moderate cytoplasmic staining in more than 50% of cells (Figs. 1 and 2).

Podoplanin was expressed in all 31 reactive mesothelial cases (100%), with a membranous pattern in 30/31 of reactive mesothelial cases (97%) and one (3%) showing cytoplasmic pattern. One case (3%) showed concomitant strong membranous and cytoplasmic staining in more than 50% of cells. More than 50% of reactive mesothelial cells were positive in 25/31 cases (80%). In 2/31 (7%) cases, there was membranous staining in 25–50% of reac-

Table II. Details of Pattern of Podoplanin Immunostaining in Adenocarcinomas and Mesothelioma in Effusions

	Pattern of podoplanin immunostaining			
	Membranous		*Cytoplasmic*	
Malignancy	*Weak*	*Moderate and strong*	*Weak*	*Moderate and strong*
Mesothelioma	4/18 (22%)	13/18 (72%)	0/18	1/18 (6%)
Reactive mesothelium	0/31	30/31 (97%)	0/31	1/31 (3%)
Lung adenocarcinoma	0/9	0/9	1/9 (11%)	0/9
Breast adenocarcinoma	0/9	0/9	2/9 (22%)	0/9
Ovarian adenocarcinoma	1/14 (7%)	0/14	0/14	0/14

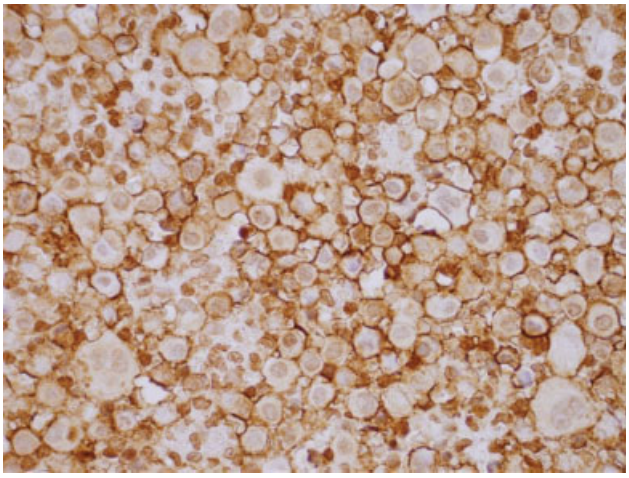

Fig. 1. Malignant mesothelioma showing moderately intense membranous staining with podoplanin (×400). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

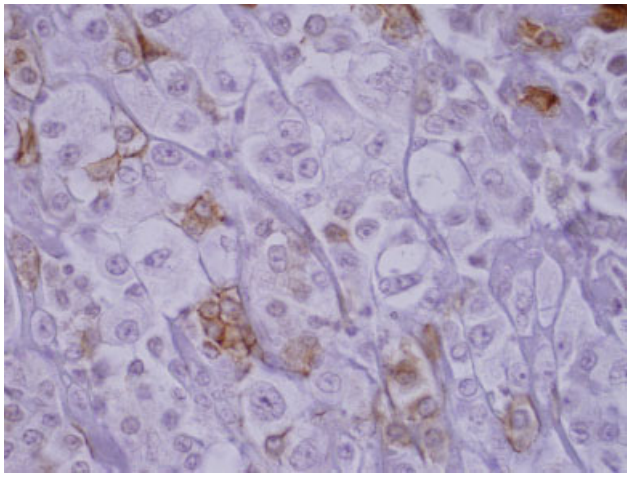

Fig. 3. Lung adenocarcinoma showing no immunoreactivity. Background mesothelial cells showing membranous podoplanin staining (×400). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

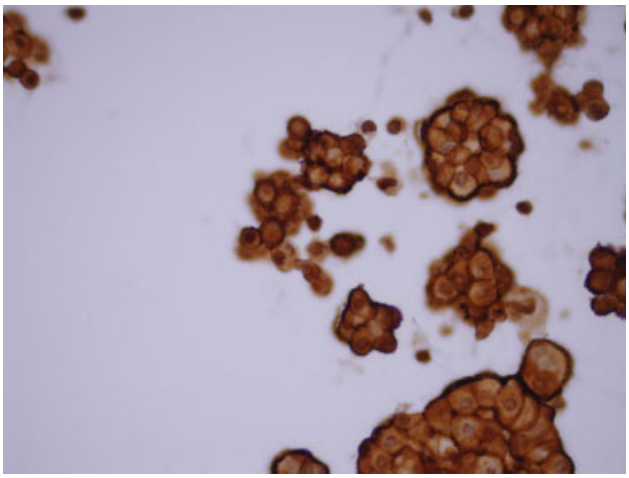

Fig. 2. Malignant mesothelioma showing strong membranous and focal moderate nuclear staining with podoplanin (×400). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

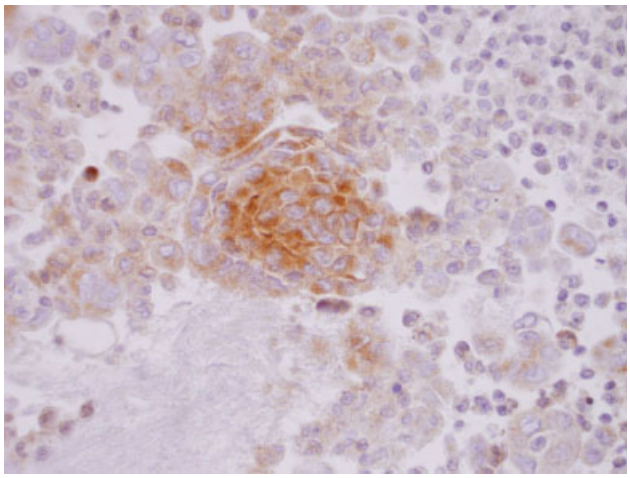

Fig. 4. Lung adenocarcinoma showing focal cytoplasmic podoplanin immunoreactivity (×400). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

tive mesothelial cells and 3/31cases (13%) had positive staining in 5–25% of cells. The intensity of membranous staining was strong in 11 cases (36%), moderate in 10 cases (32%), and weak in 10 cases (32%).

None of the lung adenocarcinoma cases (0/9) had membranous pattern staining (Fig. 3). There was weak cytoplasmic staining in 2/9 cases (22%) involving >50% of tumor cells (Fig. 4). In breast adenocarcinoma cases, none of the tumor cells showed membranous pattern (Fig. 5). In one case (11%), the tumor cells did not demonstrate any staining for podoplanin.

One of 14 cases of ovarian adenocarcinoma (7%) demonstrated weak membranous staining in less than 25% of cells (Fig. 6).

Discussion

Adenocarcinoma, particularly lung, breast, and ovarian adenocarcinomas in effusions frequently manifest as tight cell clusters and present a challenge in separating them from mesothelial cells on morphologic grounds. Immunohistochemistry is an invaluable ancillary technique in differentiating mesothelioma from adenocarcinoma.

The panel that is used to separate adenocarcinoma from mesothelioma usually includes one or more of antibodies that are reactive in mesothelioma but negative in adenocarcinoma; in addition, two or more of antibodies that are reactive in adenocarcinomas and negative or show low reactivity in mesothelioma. It has been suggested that cal-

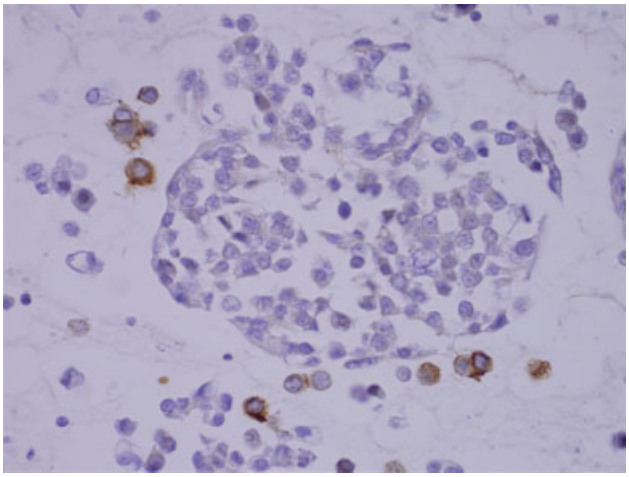

Fig. 5. Breast adenocarcinoma showing no immunoreactivity. Background mesothelial cells showing membranous podoplanin staining (×400). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

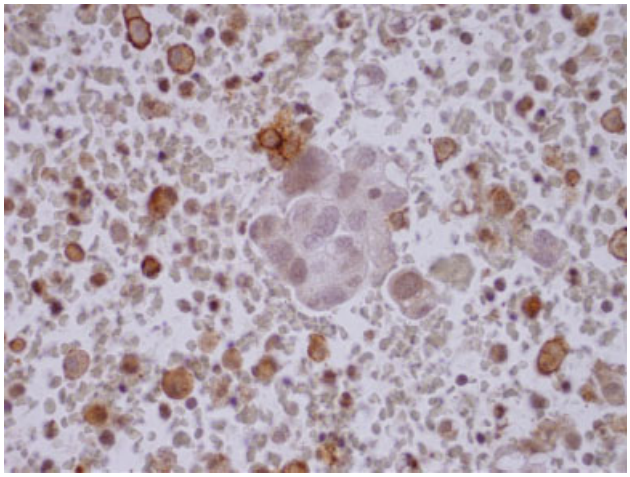

Fig. 6. Ovarian adenocarcinoma showing no immunoreactivity. Background mesothelial cells showing membranous podoplanin staining (×400). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

retinin,[19–22] CK5/CK6,[23–25] and WT1[26–29] are the best positive predictive markers for epithelioid mesothelioma and negative mesothelioma markers such as carcinoembryonic antigen, MOC-31,[30–33] Ber-EP4,[34–36] B72.3,[37,38] and CD15[39] that are commonly expressed in adenocarcinomas. However, although immunohistochemical staining has proven to be valuable in the differentiation of epithelioid mesothelioma from pulmonary or metastatic adenocarcinoma, no single antibody has demonstrated absolute sensitivity or specificity in making this distinction.[5] Consequently, there is a continuous search for a marker with higher sensitivity and specificity.

D2-40 and podoplanin are two antibodies initially recognized to stain lymphatic endothelial cells, but expression of these markers has been more recently described in mesothelial cells.[7–9] Initial reports indicate that this marker stains virtually 100% of epithelioid mesothelioma and is a highly useful discriminator between epithelioid mesothelioma and adenocarcinomas. Recent reports also indicated that 78% of sarcomatoid mesothelioma do not exhibit immunoreactivity to D2-40.[40,41]

It is worth noting that podoplanin expression is not specific to mesothelioma because it has been reported to be expressed in various other types of tumors including vascular tumors, tumors of the central nervous system (CNS), germ cell tumors, and squamous cell carcinomas.[10–13]

In this study, podoplanin is expressed in 94% of malignant mesothelioma cases with membranous pattern. The expression of podoplanin is strong in the majority of cases. Only one case showed moderate cytoplasmic expression. This is in accordance with the previous studies. About 97% (30/31) of cases of reactive mesothelium express podoplanin with membranous pattern. The intensity of podoplanin staining in reactive mesothelial cases (36% strong, 36% moderate, and 32% weak) was comparable to that observed in malignant mesothelioma (44% strong, 28% moderate, and 22% weak). Therefore, on the basis of these results, podoplanin is not a useful marker to differentiate between malignant mesothelioma and reactive mesothelial cells.

Our results demonstrate that none of the cases of lung and breat adenocarcinomas have expressed membranous podoplanin immunoreactivity (Figs. 3 and 5). However, there was cytoplasmic podoplanin immunoreactivity in 1/9 (11%) of lung adenocarcinoma and 2/9 (22%) of breast carcinoma. In these cases, the cytoplasmic immunoreactivity was weak and diffuse. Our findings require further investigation to determine the significance and reproducibility of the weak cytoplasmic podoplanin immunoreactivity in few cases of lung and breast adenocarcinomas.

In this study, 1/14 (7%) of cases of ovarian adenocarcinoma demonstrated a weak membranous immunoreactivity to podoplanin. Although it is a relatively low percentage, more cases of ovarian adenocarcinoma should be examined to determine the accurate percentage of ovarian adenocarcinoma that demonstrates membranous immunoreactivity to podoplanin.

A panel of immunohistochemistry markers is often used to differentiate mesothelioma from adenocarcinomas. Most markers are not specific for mesothelioma and cross react with other malignancies. Calretinin is a calcium-binding protein expressed in a variety of tissues including not only mesothelial cells but also adipocytes, neural tissues, and sex cord tumors. The stain is cytoplasmic, but the combination of both nuclear and cytoplasmic staining is considered more specific for mesothelial differentiation.[19–22] Calretinin stains more than 90% of epithelioid

mesothelioma and the epithelioid component in biphasic mesotheliomas. Cytokeratin 5/6 stains 65–100% of epithelioid mesothelioma and is highly useful if the differential diagnosis is essentially restricted to pulmonary adenocarcinoma versus epithelioid mesothelioma. However, CK5/6 will also stain a fairly significant percentage of breast and gynecologic malignancies and is also positive in squamous cell carcinoma.[23–25] WT-1 stains mesothelial cells in a nuclear pattern. It is positive in more than 90% of epithelioid mesothelioma but it also stains ovarian, peritoneal serous carcinomas, and small proportion of breast adenocarcinoma.[26–29] This diminishes its value when evaluating effusions in women.

In our study, podoplanin membranous immunoreactivity is highly sensitive and specific for mesothelial cells. Podoplanin stains 97% of cases of reactive mesothelial, 94% of cases of malignant mesothelioma, and 3% of all cases of adenocarcinoma (lung, breast, and ovary).

Taken together, we conclude that the membranous podoplanin immunoreactivity, in conjunction with calretinin, is superior to CK5/6 and WT-1 in differentiating epithelioid malignant mesothelioma from adenocarcinoma of the lung, breast, and ovary. However, none of them is useful in differentiating reactive mesothelial cells from epithelioid malignant mesothelioma.

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