Diagnostic Implications of Podoplanin Expression in Peripheral Nerve Sheath Neoplasms

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Abstract

By using the D2-40 antibody, we have observed podoplanin expression in Schwann cells and perineurial cells. Podoplanin expression has not been well characterized in peripheral nerve sheath tumors. Because neoplasms of neural crest lineage, including peripheral nerve sheath and melanocytic neoplasms, may share histologic and immunohistochemical characteristics, we evaluated podoplanin and S-100 expression in these lesions to determine the usefulness of podoplanin as a diagnostic marker. Diffuse podoplanin and S-100 expression was observed in 16 (76%) of 21 classical schwannomas, 6 (100%) of 6 cellular schwannomas, and 3 (75%) of 4 epithelioid malignant peripheral nerve sheath tumors (EMPNSTs). Podoplanin was expressed in 3 (7%) of 43 neurofibromas, 16 (21%) of 75 spindle cell MPNSTs (SMPNSTs), and 1 (10%) of 10 spindle cell melanomas but was absent in conventional melanoma. Only rare neurofibromas and SMPNSTs showed strong coexpression of podoplanin and S-100. These results suggest diffuse podoplanin expression or coexpression of podoplanin and S-100 is limited to schwannoma and EMPNST and may be useful in the evaluation of these neoplasms.

Podoplanin is a 38-kDa glycoprotein expressed by a variety of human cells and recognized by the monoclonal antibody D2-40.1,2 Podoplanin is a marker of lymphatic differentiation because it is expressed by normal human lymphatic endothelium but not by vascular endothelium.1-5 Podoplanin is a diagnostically useful marker for the evaluation of a variety of neoplasms, including Kaposi sarcoma,3,4,6 mesothelioma,7 testicular germ cell tumors,8 and cutaneous sebaceous neoplasms.9,10 Podoplanin is also expressed by normal mouse perineurial cells and various cells of the mouse central nervous system.1 In addition, we have observed expression of this protein in normal human Schwann cells and perineurial cells. However, to our knowledge, immunohistochemical expression of podoplanin has not been evaluated in neoplasms with schwannian or peripheral nerve sheath differentiation.

Neoplasms of neural crest lineage, including schwannoma, neurofibroma, malignant peripheral nerve sheath tumor (MPNST), and melanoma, may occasionally show overlapping histologic and immunohistochemical features, particularly S-100 protein expression.11,12 Thus, the purpose of this study was to assess podoplanin expression in neoplasms of neural crest lineage to determine if the D2-40 antibody would be useful as a diagnostic marker. Tissue microarrays (TMAs) were used to examine staining in a large number of cases.

Materials and Methods

TMAs were prepared using formalin-fixed, paraffin-embedded archival tissue from the Cleveland Clinic Foundation (Cleveland, OH), Stanford University (Stanford,
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CA), University of British Columbia (Vancouver, Canada), and University of Washington (Seattle). Duplicate 0.6-mm cores were removed from selected areas of the tissue blocks by using a tissue microarrayer (Beecher Instruments, Sun Prairie, WI). The TMAs were composed of 18 schwannomas, 7 cellular schwannomas, 43 neurofibromas, 80 spindle cell MPNSTs (SMPNSTs), 3 epithelioid MPNSTs (EMPNSTs), 10 spindle cell/desmoplastic melanomas, 4 conventional (epithelioid) cutaneous melanomas, 5 granular cell tumors, 4 perineuriomas, 32 gastrointestinal stromal tumors (GISTs), 12 solitary fibrous tumors (SFTs), and 15 cases of fibromatosis. In addition, selected complete paraffin sections were immunostained for podoplanin. These included 5 schwannomas, 6 SMPNSTs, 1 EMPNST, 13 conventional melanomas, and 1 dermal nerve sheath myxoma.

Cellular schwannoma was defined as dense fascicles of spindle cells resembling those in classical schwannoma, but with predominantly Antoni A–type areas, minimal pleomorphism and mitotic activity, and no necrosis. Of the 43 neurofibromas, 8 were solitary and localized lesions, 24 were plexiform, and 11 were diffuse type. Twenty were from patients with confirmed neurofibromatosis 1 (NF1). Low-grade SMPNST was defined as a spindle cell neoplasm with an overall appearance of a neurofibroma, focal hypercellularity with associated fascicular growth pattern, and mildly increased mitotic activity. High-grade SMPNST was defined as SMPNST without an apparent neurofibroma-like appearance and with marked mitotic activity and a diffuse fascicular growth pattern; necrosis was present in some cases. Of 56 SMPNSTs with available clinical data, 34 were from patients with confirmed NF1, 1 was from a patient with presumed NF1, and 21 were from patients confirmed not to have NF1 or presumed to have sporadic disease. Four SMPNSTs showed heterologous sarcomatous differentiation. All but 5 SMPNSTs were high grade. Of the 13 full sections of conventional melanoma, 8 were primary cutaneous lesions and 5 were metastatic neoplasms.

TMA sections were stained by using an automated stainer (Ventana Medical Systems, Tucson, AZ) with antibodies against podoplanin (D2-40, dilution 1:100; Signet, Dedham, MA) and S-100 protein (1:8,000 dilution; DAKO, Carpinteria, CA) by standard avidin-biotin peroxidase methods. Slides were scored for the percentage of positively staining cells as follows: 0, no staining; 1, 1% to 25%; 2, 26% to 50%; and 3, more than 50%. A positive result was defined as more than 25% staining in one or both cores (score 2 or 3). In general, scoring was equal in each duplicate core. In most positive cases, staining with either antibody was strong; however, in several cases, podoplanin expression was weak, as indicated in the “Results” section. Cases were excluded when one core was absent (owing to tissue exhaustion), not interpretable, or lacked cells of interest and the second core exhibited staining of 25% or fewer cells. Tumors in which one duplicate core was positive (score 2 or 3) were included regardless of the status of the second core. Cases excluded were 11 SMPNSTs, 2 schwannomas, and 1 cellular schwannoma.

Results

In our evaluation of the D2-40 antibody, we observed podoplanin expression on Schwann cells and perineurial cells in normal human peripheral nerve Image 1. The results of the immunohistochemical stains are shown in Table 1. Overall, expression of podoplanin alone or in combination with S-100 was more frequently observed in classical schwannoma, cellular schwannoma, and EMPNST compared with neurofibroma, SMPNST, spindle cell melanoma, and conventional (epithelioid) melanoma.

Of the schwannomas examined, 16 (76%) of 21 classical and all six (100%) cellular schwannomas showed strong and diffuse cytoplasmic positivity for podoplanin and S-100 Image 2A and Image 2B. In all positive cases, podoplanin expression was strong, diffuse (score, 3), and cytoplasmic Image 3A. In 2 negative classical schwannomas, podoplanin expression was weak and easily detected but expressed by 25% or fewer of the cells (score, 1). In 2 cases, there was no detectable podoplanin expression. All schwannomas examined showed diffuse cytoplasmic staining with S-100, as expected. When present, spindle cells with a perineurial

![Image 2A](https://example.com/image2a.png)

![Image 2B](https://example.com/image2b.png)

![Image 3A](https://example.com/image3a.png)
Table 1
Results of Immunohistochemical Stains in Various Neoplasms*

<table>
<thead>
<tr>
<th>Neoplasm</th>
<th>No. of Cases</th>
<th>D2-40</th>
<th>S-100</th>
<th>Combined D2-40 and S-100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schwannoma</td>
<td>21</td>
<td>16 (76)</td>
<td>21 (100)</td>
<td>16 (76)</td>
</tr>
<tr>
<td>Cellular schwannoma</td>
<td>6</td>
<td>6 (100)</td>
<td>6 (100)</td>
<td>6 (100)</td>
</tr>
<tr>
<td>Neurofibroma</td>
<td>43</td>
<td>3 (7)</td>
<td>42 (98)</td>
<td>3 (7)†</td>
</tr>
<tr>
<td>EMPNST</td>
<td>75</td>
<td>16 (21)‡</td>
<td>25 (33)</td>
<td>9 (12)§</td>
</tr>
<tr>
<td>Spindle cell melanoma</td>
<td>4</td>
<td>3 (75)</td>
<td>3 (75)</td>
<td>3 (75)</td>
</tr>
<tr>
<td>Conventional melanoma</td>
<td>10</td>
<td>1 (10)</td>
<td>9 (90)</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Granular cell tumor</td>
<td>17</td>
<td>0 (0)</td>
<td>17 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Dermal nerve sheath myxoma</td>
<td>1</td>
<td>0 (0)</td>
<td>1 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Perineuroma</td>
<td>12</td>
<td>2 (17)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Solitary fibrous tumor</td>
<td>15</td>
<td>4 (27)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>GIST</td>
<td>32</td>
<td>8 (25)</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Data are provided as number (percentage) of positive cases.
† Of the 43 neurofibromas, 1 (2%) showed strong podoplanin (D2-40) expression.
‡ Of the 75 EMPNSTs, 9 (12%) showed strong podoplanin (D2-40) expression.
§ Of the 75 EMPNSTs, 4 (5%) showed strong podoplanin (D2-40) and S-100 expression.

Image 2 A, Classical schwannoma is strongly and diffusely positive for podoplanin, highlighting the fascicular growth pattern. Like normal Schwann cells, expression is cytoplasmic. B, Cellular schwannoma is also strongly positive for podoplanin with a similar staining pattern to the classical variant. C, Epithelioid malignant peripheral nerve sheath tumor expresses podoplanin in a membranous pattern.
cell–like appearance located within the surrounding capsule strongly stained with podoplanin.

Of 4 EMPNSTs, 3 (75%) were positive for podoplanin and S-100. Expression of podoplanin was strong, diffuse (score, 3), and membranous in all cases compared with the sometimes weak staining in SMPNST. One case was negative for podoplanin and S-100.

Most SMPNSTs did not express podoplanin. Of 75 SMPNSTs, 16 (21%) showed cytoplasmic expression in more than 25% of cells, but strong intensity was present in only 9 cases (12%). In the remaining 7 positive cases, podoplanin staining was faint. Approximately one third of SMPNSTs expressed S-100. Coexpression of podoplanin and S-100 was present in 9 cases (12%). Only 4 cases (5%) showed S-100 positivity combined with strong D2-40 immunostaining intensity, however. Of the podoplanin-positive SMPNSTs, 7 were from patients with a confirmed NF1 mutation and 4 from patients with no mutation, and in 5 cases, the mutation status was unknown. In addition, 2 were histologically low-grade and 14 were high-grade neoplasms. Podoplanin expression showed no correlation with heterologous differentiation. In the majority of SMPNSTs considered negative, there was complete absence of podoplanin expression.

Few neurofibromas (3/43 [7%]) expressed podoplanin, whereas nearly all expressed S-100 protein. All podoplanin-positive cases coexpressed S-100. Although podoplanin was expressed by more than 50% of cells (score, 3) in the 3 positive cases, staining in 2 of these was faint and granular, at a level barely detectable above background. Strong cytoplasmic expression was present in only 1 case. Both weakly podoplanin-positive cases were plexiform neurofibromas from patients with confirmed NF1 status, whereas the 1 lesion with strong staining was of the diffuse type and NF1 status was unknown.

Only 1 (10%) of 10 spindle cell melanomas expressed podoplanin and S-100. One neoplasm was negative for both markers. Complete absence of podoplanin expression was observed in all 17 conventional melanomas devoid of spindle cell morphologic features. No podoplanin staining was observed in 5 granular cell tumors. Only 1 perineurioma strongly expressed podoplanin. Two perineuriomas showed weak patchy staining in a minority of cells. Subsets of spindle cell GISTs (8/32 [25%]), SFTs (2/12 [17%]), and fibromatosis (4/15 [27%]) showed strong podoplanin positivity.

Discussion

The monoclonal antibody D2-40 identifies an epitope on podoplanin, a transmembrane glycoprotein expressed by normal lymphatic endothelium but absent from vascular endothelial cells. Podoplanin is expressed by a variety of normal cells, including breast and prostate myoepithelial cells, follicular dendritic cells, basal keratinocytes of the skin and cervix (focal), type I pneumocytes, ependymal cells, and fetal cerebral germinal matrix cells. Podoplanin is also expressed in normal mouse perineurial cells and in peripheral nerves of mouse skin, tongue, and skeletal muscle. Immunohistochemical staining with D2-40 has widespread applications in diagnostic surgical pathology, including distinguishing podoplanin-positive epithelioid mesothelioma from lung adenocarcinoma, which tends to be negative. Vasiformative neoplasms such as Kaposi sarcoma, kaposi-
form hemangioendothelioma, and some angiosarcomas also express podoplanin, suggestive of at least partial lymphatic differentiation.\textsuperscript{3,4,6,17} Podoplanin is expressed in follicular and sebaceous cutaneous neoplasms,\textsuperscript{9,10} germ cell tumors,\textsuperscript{1,8,15} ependymoma, choroid plexus carcinoma, and meningioma.\textsuperscript{16,18} To our knowledge, however, podoplanin expression in peripheral nerve sheath neoplasms has not been well characterized.

Neural crest cells arise from ectodermal embryonic tissue and migrate peripherally from the area of the neural tube to form components of the peripheral nervous system, including Schwann cells and neurons and melanocytes.\textsuperscript{19,20} Neoplasms presumed to arise from neural crest progenitor cells include schwannoma, neurofibroma, MPNST, and melanoma. Although the clinical and microscopic features of these neoplasms are usually distinct, this shared derivation may sometimes lead to overlapping histologic features.\textsuperscript{11,12,21-24} For example, cellular schwannoma, cellular neurofibroma, SMPNST, and melanoma may share morphologic features, including spindle cell morphologic features, nuclear atypia, or mitotic activity.\textsuperscript{12,13,23,25,26} Psammomatous melanotic schwannoma, pigmented neurofibroma, and MPNST may each contain melanin, which is usually associated with melanocytic differentiation.\textsuperscript{21,22,27} EMPNST and conventional (epithelioid) melanoma share similar high-grade cytologic features.\textsuperscript{28}

Overlapping immunohistochemical expression patterns...
by these closely related neoplasms are also well known, most notably expression of S-100 protein. Type IV collagen is commonly expressed by classical or cellular schwannoma, although positive staining may be seen in MPNST and melanoma. Melanosome markers like HMB-45 that are usually positive in conventional melanoma and negative in most peripheral nerve sheath tumors may occasionally be positive in pigmented neurofibroma. Spindle cell melanoma often lacks expression of melanosome markers, similar to the usual immunophenotype of SMPNST. Microphthalmia transcription factor expression may be expressed by spindle cell melanoma; however, its sensitivity for spindle cell melanoma is suboptimal, and occasional schwannomas and MPNSTs express this marker. SMPNST generally lacks a specific immunophenotype and is often negative for S-100 expression. EMPNST, on the other hand, is commonly positive for S-100. Thus, additional immunohistochemical markers may aid in distinguishing these entities in some cases.

Our results suggest that podoplanin expression is relatively restricted among neoplasms of neural crest lineage. The majority of schwannomas (81%), including 76% of classical schwannomas and all cellular schwannomas, strongly expressed podoplanin. On the other hand, podoplanin expression was detected in only a minority of neurofibromas (7%), SMPNSTs (21%), and spindle cell melanomas (10%). Podoplanin positivity was often weak and patchy when present in neurofibroma and SMPNST; and strong, diffuse expression was limited to only 2% and 12%, respectively. When coexpression of podoplanin and S-100 is present, this distinction is even more apparent. In this study, 81% of classical and cellular schwannomas strongly expressed both markers, whereas only 5% of SMPNSTs showed this same combination of immunostaining. Our study found that most EMPNSTs (75%) strongly expressed podoplanin. It is important to note that no conventional melanomas expressed this protein, suggesting podoplanin may be a useful adjunct marker to distinguish these malignancies. These data agree with previously observed absence of podoplanin expression in a large number of cutaneous melanomas devoid of spindle cell morphologic features (S.S.D., unpublished data, 2007).

Additional studies are necessary to determine the usefulness of podoplanin expression in other peripheral nerve sheath tumors, including perineurioma and dermal nerve sheath myxoma. Although we found strong podoplanin expression in normal perineurial cells and cells with a perineurial cell–like appearance within the capsule of schwannoma, strong diffuse expression of this protein was identified in only 1 perineurioma. Podoplanin was not expressed in a single dermal nerve sheath myxoma.

Subsets of SFTs (17%), fibromatosis (27%), and spindled GISTs (25%) also expressed this protein. Although strong podoplanin expression is more common in schwannoma compared with these tumors that share overlapping histopathologic features, more specific markers for identification of these latter neoplasms are readily available. Finally, although not evaluated in this study, most meningiomas have been shown to express podoplanin, limiting the role of this marker in distinguishing it from schwannoma.

Like its function in normal tissues, the role of podoplanin in neoplasia is unclear. Kaposiform hemangioendothelioma, a pediatric vasoformative neoplasm, is frequently associated with adjacent dilated podoplanin-positive lymphatics. Podoplanin is expressed by squamous cell carcinoma at the site of stromal invasion, suggesting a role in cell motility and migration. The biologic basis for podoplanin expression in Schwann cells and schwannoma likewise needs to be elucidated. It is also unclear why various neoplasms with schwannian phenotypes such as schwannoma, neurofibroma, and granular cell tumor have different podoplanin expression patterns. The observed differences in podoplanin expression between schwannoma and neurofibroma may not be surprising given their distinct genetic anomalies and tumor environment. Podoplanin expression may also correlate with the apparent association of some benign and malignant nerve sheath neoplasms, namely a subset of neurofibromas that progresses to SMPNST, and the proclivity of EMPNST to arise in association with schwannoma. Relative absence of podoplanin in neurofibroma and SMPNST and consistent expression in schwannoma and EMPNST may corroborate these relationships. Finally, although granular cell tumor...
may arise in association with peripheral nerve, express S-100 protein, and share ultrastructural features of Schwann cells, its precise histogenesis is unclear. Unlike schwannoma, no granular cell tumors in our study expressed podoplanin, a phenotypic distinction between these presumably related lesions of uncertain significance.

Podoplanin expression seems to be unique to schwannoma, cellular schwannoma, and EMPNST among neoplasms of neural crest lineage. These results suggest that podoplanin expression alone or combined podoplanin and S-100 immunostaining strongly favors schwannoma over neurofibroma, SMPNST, and spindle cell/desmoplastic melanoma. Additional studies are needed to determine if podoplanin is useful for distinguishing other variants of schwannoma (ie, melanotic schwannoma) from its mimickers. Although only a small number of EMPNSTs were examined in this study, podoplanin may also aid in distinguishing it from conventional melanoma, the latter of which lacks expression of this protein.

References


