Immunohistochemical Staining for Calretinin Is Useful for Differentiating Schwannomas From Neurofibromas

Samson W. Fine, MD, Steve A. McClain, MD, and Maomi Li, MD, PhD

Key Words: Neurofibroma; Schwannoma; Calretinin; Schwann cells

DOI: 10.1309/AGBGTBRJ4W0BC7LN

Abstract

We studied 25 cases of schwannoma and 42 cases of neurofibroma immunohistochemically with antibodies to calretinin and S-100 protein to explore the potential usefulness of calretinin in differentiating schwannomas from neurofibromas. Of 25 schwannomas, 24 (96%) showed moderate to strong staining for calretinin, with the extent of staining ranging from focal to diffuse. In contrast, only 3 (7%) of 42 neurofibromas displayed focal weak to moderate staining with calretinin. All 42 cases of neurofibromas and all 25 cases of schwannomas showed diffuse moderate to strong staining with S-100 protein. Calretinin also labeled mast cells, whose presence was confirmed further by staining for c-kit, which commonly was present in both tumor types in a scattered individual cell pattern easily differentiated from the clustered pattern of neoplastic spindle cells. Taken together, these results indicate that calretinin is detected in almost all schwannomas and in only a small percentage of neurofibromas, suggesting it is a useful marker for differentiating schwannomas from neurofibromas. Although mast cells present in these 2 neoplasms also react with calretinin, the pattern of staining can be distinguished easily from that of neoplastic cells.

Schwannomas and neurofibromas are the 2 most common benign neoplasms derived from peripheral nerve. Although the early literature suggested that both were pure Schwann cell lesions, subsequent ultrastructural studies have shown that schwannomas are composed exclusively of neoplastic cells that simulate the appearance of differentiated Schwann cells, while neurofibromas display a mixture of cell types, including Schwann cells, perineurial cells, and endoneurial fibroblasts. Schwannomas typically are well circumscribed and composed of spindle cells organized as cellular areas with nuclear palisading (Antoni A) and paucicellular areas (Antoni B). Neurofibromas are less cellular and not as circumscribed as schwannomas and contain considerable extracellular myxoid material, wavy collagen fibers, and occasional neurites.

Although these tumors generally are not difficult to differentiate by standard light microscopy, in a small number of cases, they might closely resemble one another. Nuclear palisading is not present in all schwannomas, making some lesions potentially difficult to separate from cellular neurofibromas. Furthermore, schwannomas consisting exclusively of Antoni B areas are sparsely cellular and myxomatous and might mimic the histologic appearance of neurofibromas on H&E staining. The importance in distinguishing these 2 entities lies in the association of some neurofibromas with neurofibromatosis and associated syndromes.

Immunohistochemical staining for S-100 protein has been used as an adjuvant marker in the differential diagnosis of schwannoma and neurofibroma. Although the percentage of positive cells and the intensity of staining usually are higher in schwannomas, S-100 might not reliably distinguish the 2 neoplasms. Calretinin, a calcium-binding protein...
belonging to the same protein family as S-100,7 is expressed primarily in cells of the central and peripheral nervous systems.8,9 We have previously shown calretinin to be a useful marker for granular cell tumors,10 neoplasms considered to be of schwannian origin,11 suggesting the value of calretinin in identifying Schwann cell lesions. In the present study, we evaluated and compared the expression of calretinin with that of S-100 to determine the usefulness of calretinin in distinguishing these 2 common neoplasms.

Materials and Methods

Formalin-fixed, paraffin-embedded tissue blocks of schwannomas (25 cases), neurofibromas (42 cases), and nonneoplastic tissue samples containing peripheral nerves (8 cases) were selected from the surgical pathology archives of Montefiore Medical Center, Bronx, NY. All sections were deparaffinized, rehydrated, and quenched with hydrogen peroxide. Antigen retrieval was performed as follows: for S-100, calretinin, and c-kit stains, slides were incubated with DAKO Epitope Buffer (DAKO, Carpinteria, CA) in a steam bath at 95°C for 45 minutes. After equilibration in phosphate-buffered saline for 15 minutes, the slides were placed in an autostainer (DAKO) and stained with antibodies to S-100 protein (polyclonal, 1:4,000 dilution; DAKO), calretinin (polyclonal, 1:50 dilution; Zymed, San Francisco, CA), and c-kit (polyclonal, 1:50 dilution; DAKO). Immunoreactivity was detected using DAKO EnVision methods (DAKO) according to manufacturer-recommended procedures. For negative control experiments, slides were treated with the same procedure, including antigen retrieval, except for replacement of primary antibodies with a negative diluent (Zymed). Immunoreactivity was evaluated for intensity of labeling (−, 1+, 2+, and 3+) and percentage of cells stained (<25%; 25%-75%, and >75%).

Results

We studied 25 cases of schwannoma and 42 cases of neurofibroma. The schwannomas were from soft tissue of head and neck (2 cases), upper extremity (10 cases), lower extremity (4 cases), back (2 cases), retroperitoneum (3 cases), and parotid gland, vulva, pleura, and stomach (1 case each). The neurofibromas came from skin and soft tissue of head and neck (5 cases), upper extremity (7 cases), lower extremity (6 cases), back (11 cases), trunk (11 cases), and perineum (1 case); 1 lesion originated in the pleura.

As shown in Table 1, 24 (96%) of 25 cases of schwannomas displayed 2+ to 3+ staining intensity with calretinin. Of the 24 labeled cases, 8 (33%) had fewer than 25% of tumor cells stained, 13 (54%) had 25% to 75% of tumor cells stained, and 3 (13%) had more than 75% of tumor cells stained. Both cytoplasmic and nuclear stains for calretinin were noted. In some cases, Antoni A areas and Antoni B areas were labeled equally Image 1A and Image 1B, while in others, staining was limited more to Antoni A or Antoni B areas Image 1C. When the staining was present only focally, the positively stained cells usually were clustered together (Image 1C). In contrast with schwannomas, only 3 (7%) of 42 cases of neurofibroma were immunohistochemically positive for calretinin Image 2A. The 3 labeled cases each displayed 1+ to 2+ staining in fewer than 25% of neoplastic cells.

All 25 cases of schwannoma (100%) were positive for S-100 protein. In all cases, staining was detected in more than 75% of cells, and 24 of 25 cases showed strong intensity (3+) Image 3A. All 42 cases of neurofibroma also were positive for S-100 protein, with moderate to strong intensity (2+ to 3+) in more than 75% of cells in 23 cases and in 25% to 75% of cells in 19 cases Image 3B. In all schwannomas and neurofibromas, calretinin labeled mast cells in a scattered individual cell pattern Image 4A, in contrast with the clustered pattern of neoplastic spindle cells (Image 1C). The nature of mast cells was confirmed further by their positive reactivity with c-kit Image 4B, which did not label the tumor cells of schwannomas or neurofibromas. The number of mast cells, in general, was greater in neurofibromas than in schwannomas.

Peripheral nerve bundles showed variable reactivity for calretinin. Among the 8 blocks of nonneoplastic tissue stained with calretinin, 3 blocks, including 2 from skin and 1 from soft tissue of leg, lacked staining in the nerve bundles, while 2 (retroperitoneum and parotid area) were stained strongly Image 5A. In the remaining 3 (sciatic nerve, periprostatic soft tissue, and soft tissue of leg), the nerve bundles were stained very focally Image 5B. In addition to

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Immunohistochemical Results for Calretinin and S-100 Protein in Neurofibromas and Schwannomas</strong></td>
</tr>
<tr>
<td>Neoplastic Cells Stained</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Schwannoma</td>
</tr>
<tr>
<td>&lt;25</td>
</tr>
<tr>
<td>25-75</td>
</tr>
<tr>
<td>&gt;75</td>
</tr>
<tr>
<td>Neurofibroma</td>
</tr>
<tr>
<td>&lt;25</td>
</tr>
<tr>
<td>25-75</td>
</tr>
<tr>
<td>&gt;75</td>
</tr>
</tbody>
</table>

* Expressed as number of positive cases/number of total cases (percentage).
† Moderate to strong (2+ to 3+) staining intensity in all positive cases.
‡ Among the 3 positive cases, 1 had weak (1+) and 2 had moderate (2+) staining intensity.
Immunohistochemical analysis for calretinin in schwannomas. **A**, An Antoni A area of a schwannoma (H&E, ×200). **B**, The same area displays strong diffuse staining for calretinin (×200). **C**, In a different schwannoma, only the Antoni B area, not the Antoni A area, is stained. Note the clustered pattern of staining (calretinin, ×200).

Immunohistochemical analysis for calretinin in neurofibroma. **A**, Neurofibroma (H&E, ×200). **B**, Immunohistochemical stain of the same tumor labels scattered mast cells, whereas the tumor cells are negative (calretinin, ×200).
axons, some Schwann cells were labeled positively as judged by the spindly shape of the stained cells (Image 5B).

Discussion

Calretinin is a 29-kd, calcium-binding protein belonging to the family of EF-hand proteins that includes S-100 protein. The EF-hand proteins are characterized by a helix-loop-helix structure, which functions as the calcium-binding site.\(^7\) Calretinin is expressed primarily in certain types of neurons in the central and peripheral nervous systems.\(^8,9\) Extraneuronal expression of calretinin has become a topic of interest in recent years. In 1996, Doglioni et al\(^{12}\) demonstrated that calretinin was a marker of human mesothelial cells and mesothelioma. Subsequently, others have shown the usefulness of calretinin for differentiating mesothelioma from adenocarcinoma,\(^{13,14}\) for identifying certain types of ovarian epithelial and stromal cells,\(^{15-17}\) and as a marker for some types of ovarian sex–cord stromal tumors,\(^{15-17}\) Leydig cell tumors of the testis,\(^{18,19}\) adrenal cortical tumors,\(^{20}\) and adenomatoid tumors.\(^{19,21}\) Although calretinin is known to be expressed in peripheral neural tissues,\(^{8,9}\) its presence in the tumors of peripheral nerves has not been well studied.
Schwannomas and neurofibromas arise from peripheral nerves. Schwannomas consist almost exclusively of Schwann cells, while neurofibromas contain several cellular components, including Schwann cells, perineurial-like cells, and endoneurial fibroblasts. Although a vast majority of these neoplasms are distinguishable by their pattern of growth and cellular composition, differential diagnosis might be difficult in a small number of cases. Some schwannomas might not display the characteristic feature of nuclear palisading, making it potentially difficult to separate schwannomas from cellular neurofibromas. Furthermore, schwannomas predominantly composed of Antoni B areas might mimic the histologic appearance of neurofibromas.

Several immunohistochemical markers have been studied for their potential use in the differential diagnosis of schwannomas and neurofibromas. S-100 protein is expressed in peripheral Schwann cells, and immunohistochemical staining for S-100 protein has been used as an adjunct in the differential diagnosis of peripheral nerve neoplasms, not only for separating them from tumors of nonneural origin but also for differentiating schwannomas from neurofibromas, the latter of which is based on the fact that staining is more uniform and pronounced in schwannomas than in neurofibromas.\(^5\)\(^,\)\(^22\) However, the difference in the number of stained cells and the staining intensity is not always reliable, particularly in dealing with individual cases. Other markers include factor XIIIa,\(^5\)\(^,\)\(^23\)\(^,\)\(^24\) Leu-7,\(^25\)\(^,\)\(^26\) myelin basic protein,\(^25\)\(^,\)\(^27\)\(^,\)\(^28\) glial fibrillary acidic protein,\(^25\)\(^,\)\(^29\) epithelial membrane antigen, and Glut-1.\(^30\) However, owing to their variably reported specificity and sensitivity, none of these markers is used widely.

Recently, we have shown the usefulness of calretinin expression as a marker for granular cell tumors,\(^10\) neoplasms believed to be derived from Schwann cells.\(^11\)\(^,\)\(^31\)\(^-\)\(^34\) In the present study, we demonstrated that calretinin staining was positive in 24 (96%) of 25 schwannomas. Although the number of cells stained varied from focal (<25%) to diffuse (>75%), the staining intensity was moderate to strong in all positively stained cases. In contrast, only 3 (7%) of 42 neurofibromas were stained with calretinin, with weak or moderate intensity in fewer than 25% of the tumor cells. These results strongly suggest that calretinin is a useful marker for differentiating schwannomas from neurofibromas. Our results also demonstrate that calretinin is superior to S-100 protein, the latter of which was positive in all schwannomas and neurofibromas. Although as a group, the percentage of positive tumor cells and staining intensity for S-100 protein was slightly higher in schwannomas than in neurofibromas, this difference could not be applied reliably to individual cases. We, therefore, recommend that calretinin replace or at least be used in combination with S-100 protein for differentiating schwannomas from neurofibromas.

The presence of mast cells in benign nerve sheath tumors has been recognized for some time. Early studies\(^35\) showed a high concentration of diffusely distributed mast cells in neurofibromas and fewer, often restricted to Antoni B areas, in schwannomas. Both c-kit (CD117) and calretinin have been detected immunohistochemically in mast cells. Strong membrane reactivity for c-kit has been used to differentiate systemic mast cell disease from its mimickers.\(^36\)\(^,\)\(^37\) Mangini et al\(^38\) found calretinin to be a sensitive marker for

---

**Image 5** A peripheral nerve from the parotid area (A) shows strong immunoreactivity with calretinin, whereas a nerve from a leg (B) is stained only focally. Some positively stained spindly cells (B) are morphologically consistent with Schwann cells (A and B, calretinin, ×200).
mast cell lesions of the skin. Our results that calretinin positively labeled mast cells in both schwannomas and neurofibromas further support these previous findings. In our present study, as in earlier studies, mast cells were observed more frequently in neurofibromas than in schwannomas. In both tumor types, the mast cells were present in a scattered individual cell pattern, which could be differentiated easily from the clustered pattern of the neoplastic cells. These scattered mast cells also were stained immunohistochemically by c-kit, which did not react with the neoplastic cells of neurofibromas or schwannomas. Although we did not find difficulty in differentiating calretinin-stained mast cells from neoplastic cells, we recommend that an anti–c-kit stain be performed for cases in which a distinction is difficult, because mast cells are positive for both calretinin and c-kit, while neoplastic Schwann cells are negative for c-kit.

It was to our surprise that although both tumor types are derived from Schwann cells, calretinin was detected in almost all cases of schwanna, in contrast with a very small percentage of neurofibromas. We offer 2 possible mechanisms to explain this finding.

First, schwannomas and neurofibromas might originate from different types of Schwann cells. Little is known about the expression of calretinin in Schwann cells; however, several studies using animal tissue have revealed that calretinin is expressed in some types of but not all preganglionic and postganglionic neurons, including their axons and nerve terminals. Our findings also indicate a variable degree of calretinin expression in peripheral nerves. The staining pattern (Images 5A and 5B) suggests that in addition to axons, calretinin also labeled some Schwann cells. It may be speculated that the expression status of calretinin in Schwann cells is related closely to that of the neurons the Schwann cells interact with, and, therefore, schwannomas and neurofibromas might originate from calretinin-positive and calretinin-negative Schwann cells, respectively.

Second, the difference in calretinin reactivity might be the result of different pathogenetic pathways associated with schwannomas and neurofibromas. Neurofibromas typically are associated with abnormalities of the NF1 gene, whereas schwannomas are associated with NF2 gene abnormalities. Furthermore, Serra et al, studying NF1 mutations using a cell culture system, found that in neurofibromas with NF1 mutations, Schwann cells but not the other cells harbored mutations at the NF1 locus. They also obtained 2 genetically distinct subpopulations of Schwann cells, only one of which showed mutations in both NF1 alleles. Their results indicate that even within a neurofibroma, genetically distinct populations of Schwann cells exist. We speculate that different pathogenetic mechanisms might result in varying expression of certain genes, including calretinin. Further studies are required to better understand the difference in the expression of calretinin between schwannomas and neurofibromas.

The only case of calretinin-negative schwannoma in the present study arose from the stomach. This tumor also was negative for c-kit, excluding the possibility of a gastrointestinal stromal tumor. It is notable that recently, Lasota et al found a markedly diminished frequency of NF2 mutations in schwannomas of the gastrointestinal (GI) tract compared with conventional schwannomas from other sites, suggesting that GI schwannomas might represent a genetically distinct group of peripheral nerve sheath tumors. The absence of calretinin staining in our gastric schwannoma might reflect underlying differences of GI schwannomas from schwannomas originating from other anatomic sites.

The results of the present study indicate that immunohistochemical staining for calretinin is useful for differentiating schwannomas from neurofibromas. Furthermore, the pattern of calretinin staining suggests that schwannomas and neurofibromas might originate from distinct Schwann cell types, or different pathogenetic pathways involving the 2 types of tumors might result in differences in gene expression, including calretinin. Although mast cells present in both tumors reacted with calretinin, the pattern of staining could be distinguished easily from that of neoplastic cells. Whether calretinin would be helpful for discriminating other peripheral nerve sheath tumors, such as cellular schwannomas and malignant peripheral nerve sheath tumors, remains a topic for future study.

From the Department of Pathology, Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, NY.

Presented in part at the United States and Canadian Academy of Pathology Annual Meeting, Vancouver, Canada, March 6-12, 2004.

Address reprint requests to Dr Li: Dept of Pathology, Albert Einstein College of Medicine and Montefiore Medical Center, 111 E 210th St, North 4 Silver Zone, Bronx, NY 10467.

Acknowledgments: We thank Ernestine Middleton, Harold Jones, Musa Ali, and Narasimha Ranginani, Montefiore Medical Center; Division of Histology, Dermatopathology, and Immunohistochemistry, for their expert technical assistance.

References


34. Mukai M. Immunohistochemical localization of S-100 protein and peripheral nerve myelin proteins (P2 protein and P0 protein) in granular cell tumors. *Am J Pathol.* 1983;112:139-146.


