Differentiating Neurotized Melanocytic Nevi From Neurofibromas Using Melan-A (MART-1) Immunohistochemical Stain

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**Context.**—Neurotized melanocytic nevi and neurofibromas are common, benign cutaneous neoplasms. Usually they are histologically distinct from each other; however, neurotized melanocytic nevi and neurofibromas can be clinically and histologically similar.

**Objective.**—To determine whether Melan-A (MART-1) immunohistochemical stain is sufficient to differentiate neurotized melanocytic nevi from neurofibromas.

**Design.**—Forty-nine consecutive specimens of melanocytic nevi with neurotization and 49 specimens of neurofibromas were selected. We used antibodies against Melan-A, S100, and neurofilament protein.

**Results.**—All of the melanocytic nevi showed Melan-A staining within the neurotized areas, with most of the areas staining strongly positive, whereas all the neurofibromas were completely absent of Melan-A stain. All of the nevi, including the neurotized areas, stained strongly and diffusely for S100, whereas all the neurofibromas showed a distinctive, sharp, wavy pattern of S100 staining. Neurofilament protein showed scattered staining of both melanocytic nevi and neurofibromas.

**Conclusions.**—Our data indicate that Melan-A immunohistochemical staining is helpful in differentiating neurotized melanocytic nevi from neurofibromas when distinction on histomorphology alone is difficult.

which are encoded by 3 different genes located at band 8p21 (NEFL and NEFM) and band 22q12.2 (NEFH). Antibodies to neurofilament protein, which presumably stain the intratumoral axons in neurofibroma, stain positive in benign nerve sheath tumors but are not useful in differentiating neurofibromas from Schwannomas. Because neurofibromas contain Schwann cells and frequent nerve twigs, we hypothesize that antibodies to neurofilament protein may be supplemental to Melan-A in differentiating neurofibromas from neurotized melanocytic nevi.

MATERIALS AND METHODS

Forty-nine consecutive specimens of partially neurotized melanocytic nevi (43 patients) and 49 consecutive specimens of neurofibromas (43 patients) were selected from the surgical pathology archives at Washington University School of Medicine (St Louis, Missouri) from 2006 through 2008. The cases with little material left in the blocks were excluded. The archival slides stained with hematoxylin-eosin were reviewed by 3 pathologists independently. Additionally, 5-μm sections were taken from the paraffin blocks and stained using antibodies against Melan-A (prediluted, monoclonal, catalog number 790-2990; Ventana Medical Systems, Tucson, Arizona), S100 protein (prediluted, polyclonal, catalog number 760-2523; Ventana Medical), and neurofilament (prediluted, clone 2F11, monoclonal, catalog number 760-2661; Ventana Medical) following the manufacturer’s protocols in a BenchMark XT automated slide stainer (Ventana Medical). In brief, heat-induced epitope retrieval with ethylenediaminetetraacetic acid–Tris base buffer (pH 8.0; catalog number 950-124; Ventana Medical) was used for Melan-A immunohistochemical stain; primary antibody incubation time was 16 minutes; an Ultra View Universal alkaline phosphatase red detection kit (catalog number 760-501; Ventana Medical) was used for Melan-A and an Ultra View Universal DAB detection kit (catalog number 760-500; Ventana Medical) was used for S100 and neurofilament.

RESULTS

Patient Demographics and Histopathology

Of the 49 specimens of neurotized melanocytic nevi (Figure 1, A and B), 28 (57%) were from the trunk, and 21 (43%) were from the head and neck region. Nine (18%) were compound melanocytic nevi, and 36 (73%) were intradermal melanocytic nevi. The patient population was composed of 14 men and 29 women, and the median age was 40 years (range, 14–84 years). Neurotized areas of the melanocytic nevi ranged from less than 25% to greater than 75% of the entire lesion, with most lesions (34 of 45; 76%) composed of less than 50% neurotized areas. All nevi showed congenital features, except the 4 specimens mentioned below. Rare individual or patchy, pigmented melanocytes and melanophages were seen in 26 specimens (53%), most of which were superficially located. Pigment was associated with neurotized areas in only 4 specimens (8%), all of which was very focal. Fatty infiltrates were seen in 10 cases (20%) and all were seen in association with the neurotized areas.

Four biopsies, diagnosed previously as melanocytic nevi with near-complete neurotization, were included among the melanocytic nevi. Upon reevaluation of the hematoxylin-eosin slides, a diagnosis of neurofibroma was suspected before immunohistochemical staining. Of the 49 specimens of neurofibromas (Figure 2, A and B), 33 (67%) were from the trunk, 8 (16%) from the head and neck region, and 8 (16%) from the upper (n = 7; 14%) and the lower extremities (n = 1; 2%). The patient population was composed of 17 men and 26 women, and the median age was 56 years (range, 32–84 years). All neurofibromas were well-circumscribed, nonencapsulated, dermal or subcutaneous neoplasms. There was a diffuse growth of loosely arranged, wavy spindle cells in a pale-staining stroma. Increased small-caliber ectatic vessels and mast cells were present in the stroma. Fatty infiltrates were seen in 9 cases (18%). No plexiform features were noted.

Immunohistochemistry

Forty-five of the melanocytic nevi (92%) showed Melan-A staining within the neurotized areas. In most cases (42 of 45; 93%), the neurotized areas showed strong positive signal intensity (Figure 3, A through C). Only 3 (7%) of the cases showed a weak signal and stained in less than 25% (n = 2; 4%) or between 25% and 50% (n = 1; 2%) of the neurotized areas. Fifteen cases (33%) showed positive staining of more than 75% of the neurotized areas; 12 cases (27%) showed staining of 25% to 75% of the neurotized areas, 9 cases (20%) had 25% to 50% staining, and 9 cases (20%) showed less than 25% staining. None of the neurofibromas (0 of 49; 0%) stained for Melan-A. Normal numbers of Melan-A+ melanocytes were observed in the overlying epidermis in all cases of neurofibromas (Figure 3, D).

All of the melanocytic nevi, including the neurotized areas, stained strongly and diffusely for S100 with an intense, coarse, and globoid appearance (Figure 4, A and B). The cytoplasm stained more diffusely than the nuclei. The neurofibromas showed a distinctive, sharp, wavy pattern of staining (Figure 5, A and B), with mostly cytoplasmic and some nuclear pattern. The 4 cases (8%) previously diagnosed as melanocytic nevi but suspected to be neurofibromas after review of routine stains (Figure 6, A and B), showed complete absence of Melan-A staining and the S100 pattern of staining was similar to the other neurofibromas (Figure 6, C and D).

Neurofilament protein antibody stained almost all cases of neurofibromas (48 of 49; 98%), with occasional cytoplasmic positivity in both nerve twiglike areas and random, single cells; about half of the cases of neurotized melanocytic nevi (25 of 49; 51%) showed occasional cytoplasmic positive staining within random tumor cells. The entrapped, normal nerve fibers within both tumors all stained positively.

COMMENT

Melanocytic nevi and neurofibromas both originate from neural crest-derived stem cells. These stem cells are capable of differentiating into components of the peripheral nervous system, including axons and Schwann cells, somatic, and autonomic nervous systems. They can also differentiate into various other cell types including, neuroendocrine and amine precursor uptake and decarboxylation cells, melanocytes, mesenchymal cells, and chondrocytes. Neurotization of melanocytic nevi refers to the presence of elongated or slender melanocytes (exaggeration of the type C melanocytes) with fibrillar cytoplasm, often in the mid to deep portions of some nevi. The melanocytes within neurotized areas are arranged in short bundles or structures resembling Meissner corpuscles or Verocay bodies. Some authors prefer to use the term melanotic nevus with peripheral nerve...
sheath differentiation. Given the histologic and immunologic similarities to neuroid tissue, type C melanocytes were initially thought to result from metaplasia or maturation of melanocytes into Schwannian cells with acquisition of a peripheral nerve phenotype. However, most current evidence supports that type C cells are melanocytes rather than Schwann cells.

Melanocytic nevi are often clinically distinct from neurofibromas because of the presence of pigment. However, pigment has been reported in neurofibromas, and senescent nevi are often nonpigmented. Thus, melanocytic nevi are commonly included in the clinical differential diagnosis of neurofibromas. The distinction under the microscope may also pose a diagnostic challenge. The most common scenario is when the specimens are small and the melanocytic nevi exhibit large areas of neurotization. In addition, pigmented neurofibromas have been reported, and small melanocytic nevi have been shown to be associated with neurofibromas within the same specimens in neurofibromatosis, type 1. In our study, the following histologic features were useful in distinguishing between these 2 entities: (1) neurotized melanocytic nevi had congenital and nested growth patterns, type A and type B melanocytes, in the nonneurotized areas and pigment; and (2) neurofibromas had diffuse growth patterns with the characteristic feature of more ectatic, small-caliber vessels. A fatty infiltrate was noted in similar numbers of neurotized melanocytic nevi (10 of 45; 22%) and neurofibromas (9 of 53; 17%). Thus, we consider fatty infiltrate to not be a useful feature in distinguishing these 2 entities.

Several previous studies have mainly used neurilemmal markers to differentiate these 2 entities. Type C melanocytes showed identical staining pattern when compared with type A and type B melanocytes using S100 protein, Leu-7, glial fibrillary acid protein, and myelin-basic protein. Melan-A, a marker of melanocytic differentiation, has been established in routine clinical use to label melanocytes specifically but has not yet, to our knowledge, been evaluated in the literature as useful in differentiating neurotized melanocytic nevi from neurofibromas. There is a misconception that Melan-A stains negative in neurotized melanocytic nevi, but it has been evaluated in only a handful of cases and showed mixed...
results. Our study demonstrated positive Melan-A staining in all melanocytic nevi with partial neurotization. About 60% of the cases showed more than 50% of the neurotized areas staining positive for Melan-A. Scattered, positively stained melanocytes were usually seen throughout the neurotized area. None of the neurofibromas were positive for Melan-A. We used a red chromogen for immunohistochemistry detection, thus, eliminating the possibility of false-positive staining of pigmented melanophages or dendritic cells. In our opinion, any Melan-A+ dermal cells support a diagnosis of a neurotized melanocytic nevus rather than a neurofibroma.

Although most of the neurotized areas in melanocytic nevi stained for Melan-A, there were areas devoid of staining. In congenital melanocytic nevi, the area without detectable Melan-A staining can be large (D. Lu MD, PhD,

Figure 3. Neurotized melanocytic nevi (n = 3) with positive Melan-A staining of different neurotized areas. A through C, Most neurotized melanocytes show strong Melan-A staining. D, Neurofibroma with Melan-A staining absent and positively stained intraepidermal melanocytes (original magnifications ×100).

Figure 4. Neurotized melanocytic nevus with a coarse, globoid, S100 staining pattern (original magnifications ×100 [A] and ×400 [B]).

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unpublished data, February 2011). If a small biopsy was taken from those areas only, the negative Melan-A staining could be misleading. This may be the reason that previous reports\textsuperscript{12,14} stated that neurotized melanocytic nevi were negative for Melan-A. Positive S100 protein staining has been demonstrated in both neurotized melanocytic nevi and neurofibromas.\textsuperscript{2,4,30} However, the different staining pattern has not been specified between these 2 entities. Our study showed an intense, globoid staining pattern of S100 protein among the neurotized areas as in the previous report.\textsuperscript{2} There is a distinctive fine, sharp, and wavy staining pattern in all the neurofibromas. Thus, when a differential diagnosis of melanocytic nevus with complete neurotization and neurofibroma is
proposed in a small biopsy, characterizing the S100 staining pattern may aid in the diagnosis if the area lacks Melan-A staining.

Neural twigs are frequently present in neurofibromas, but whether these axonlike structures are present in neuromatized melanocytic nevi is not well known. Our immunofluorescent antibody detected scattered positive cells in neuromatized melanocytic nevi as well as in neurofibromas without morphologically identifiable neural twigs. This finding is similar to that of previous studies and supports immunofluorescent protein not being a useful antibody to aid in the differential diagnosis between neuromatized melanocytic nevi and neurofibromas.

In our study, Melan-A was expressed in virtually all melanocytic nevi, with generally strong staining retained in areas of neurotization, whereas all cases of neurofibromas were negative for Melan-A staining. Additionally, neuromatized melanocytic nevi and neurofibromas showed a distinctive S100 staining pattern. Both neuromatized melanocytic nevi and neurofibromas showed similar occasional staining for neurofilament. Thus, Melan-A immunohistochemical staining can be helpful in differentiating neuromatized melanocytic nevi from neurofibromas in cases in which it is difficult to distinguish between them based on histomorphology alone. S100 protein staining pattern can also be used to aid in this distinction. In contrast, neurofilament immunohistochemical stain is not helpful in this matter.

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References