Neuropilin-2 as a useful marker in the differentiation between Spitzoid malignant melanoma and Spitz nevus

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Background: Spitzoid malignant melanoma (SMM) shares many histopathologic features with Spitz nevus (SN). The distinction between SMM and SN remains one of the most difficult diagnostic problems in dermatopathology. Neuropilin-2 (NRP2) is a cytoplasmic/cell surface protein that is a mediator of melanoma-endothelial cell interaction.

Objective: The aim of this study was to evaluate NRP2 expression in SMM and SN and to determine whether it can reliably differentiate between the 2 groups.

Methods: We studied the expression of NRP2 in 19 cases of SMM and 19 cases of SN from Yale Spitzoid Neoplasm Repository, New Haven, Conn.

Results: All 19 cases of SMM (100%) expressed NRP2. Most SMM showed moderate- and high-intensity staining in the majority of the melanoma cells. Most of the SN (14/19, 74%) were negative for the marker. NRP2 labeled only 5 of 19 SN (26%) and all of them demonstrated mild staining intensity. $NRP2^{+}$ staining was statistically significant in differentiating SMM from SN ($P < .05$).

Limitations: Small study size is a limitation.

Conclusions: NRP2 expression in SMM and SN may be a useful adjunct marker, in addition to histopathologic evaluation, in the differentiation between these 2 entities. (J Am Acad Dermatol 2013;68:129-37.)

Key words: immunohistochemical staining; melanoma; neuropilin; Spitz nevus; Spitzoid malignant melanoma.

Spitzoid malignant melanoma (SMM) is a variant of malignant melanoma (MM), which shares many clinical and histopathologic features with Spitz nevus (SN). In some cases, SMM may be difficult, if not impossible, to distinguish from SN histologically, and it is one of the most difficult lesions to diagnose in dermatopathology.[1] In many cases the diagnosis of SMM was made after tumors, originally diagnosed as SN, subsequently led to adverse events such as recurrence, lymph node metastases, distant metastases, or death.[2-4]

Despite the fact that this subject has been extensively studied for more than 60 years after the original description of "melanomas of childhood" by Sophie Spitz,[5] there is no single set of reliable histologic criteria that can definitively separate SN from SMM.[6,7] Suggested features in favor of SMM over SN are: large size (>1.0 cm), tumor extension

Abbreviations used:

MM:	malignant melanoma
NRP:	neuropilin
SIS:	staining intensity score
SMM:	Spitzoid malignant melanoma
SN:	Spitz nevus

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into the subcutis, presence of ulceration, high mitotic index, presence of multiple mitoses at the base of the lesion and atypical mitoses, prominent upward epidermal spread of tumor cells, and lack of maturation at the base.[8,9] Researchers have also attempted to differentiate SMM from SN by using immunohistochemical, molecular, and proteomic methods.

Neuropilins (NRPs) are co-receptors for class 3 semaphorins, which are polypeptides with key role in axonal guidance, and are also co-receptors for members of the vascular endothelial growth factor family of angiogenic cytokines.[10,11] NRP1 and NRP2 are expressed by a wide variety of human tumor cell lines and diverse human neoplasms. They have been implicated in mediating the effects of vascular endothelial growth factors and semaphorins on the proliferation, survival, and migration of cancer cells.[12,13] NRP2 plays a major role in the development of normal lymphatic vasculature.[14] Recent studies suggest that blocking of NRP2 binding to vascular endothelial growth factor-C inhibits tumor cell metastasis.[15] NRP2 has recently been shown to have a critical role in melanoma-endothelial interactions and lymphangiogenesis of MM.[16,17] Based on the expression of NRP2 in cutaneous MM we hypothesized that NRP2 could be a potential biomarker to distinguish SMM from SN.

CAPSULE SUMMARY

- Neuropilin-2 is a cytoplasmic/cell surface protein that is a mediator of melanoma-endothelial cell interaction.
- All Spitzoid malignant melanomas in our study showed diffuse moderate to strong expression of neuropilin-2 whereas the majority of Spitz nevi were negative.
- Neuropilin-2 expression may be a useful adjunct marker, in addition to histopathologic evaluation, in the differentiation between Spitzoid malignant melanomas and Spitz nevi. It might be particularly helpful in the diagnosis of challenging atypical Spitzoid neoplasms.

METHODS

Case selection

After institutional review board approval, 19 cases of SMM and 19 cases of SN were chosen retrospectively from the Yale Spitzoid Neoplasm Repository, New Haven, CT. The diagnoses of SMM and SN were verified by the authors according to established histologic criteria.[3,18] The study included histologically unequivocal SN and primary cutaneous SMM, which were diagnosed initially by a board-certified dermatopathologist. The majority of these cases were seen by multiple dermatopathologists at consensus conference at the time of the initial diagnosis. In addition, all cases chosen for the study underwent blind confirmatory review by 2 other dermatopathologists from the Yale Dermatopathology Laboratory.

Staining for NRP2

Immunohistochemical studies were performed on 4-μm formalin-fixed paraffin-embedded tissue sections using an autostainer (Dako, Carpinteria, CA). The slides were deparaffinized and rehydrated using a graded alcohol series. Citrate buffer (pH 6.0, 10 mmol/L) was used for antigen retrieval. Using the capillary gap method, the sections were incubated for 15 minutes with rabbit polyclonal antibodies against NRP2 (SC-5542, Santa Cruz Biotechnology, Santa Cruz, CA). A dilution of 1:50 was used to provide the optimum staining results. Diaminobenzidine with LSAB2 detection system (Dako catalog no. K0675) was used as chromogen and the sections were counterstained with hematoxylin. Appropriate negative and positive controls were included. NRP2 staining is normally seen in liver, kidney, fallopian tubes, pancreas, placental tissue, testis, prostate, striated muscle cells, and suprabasal epidermis.[16] We used suprabasal epidermis as an internal positive control for NRP2.

Scoring of NRP2 expression

All cases were reviewed and scored for the presence of NRP2 expression independently and blindly by 3 of the authors (J. W., A. R. M., and R. L.). For cases with discrepancy, a consensus was achieved. The staining results were reported as a staining intensity score (SIS) on a scale of 0 to 9 (1-3 = low; 4-6 = moderate; 7-9 = high). SIS was calculated by multiplying a score for staining intensity (0 = negative, 1 = weak, 2 = moderate, 3 = strong) by a score of 0 to 3 of the percentage of cells staining: 0 (none), 1 (1%-33%), 2 (34%-66%), and 3 (67%-100%).

RESULTS

The clinical and histopathologic characteristics of SMM and SN are summarized in Tables I and II. Patients with SMM were all adults and ranged from 29 to 75 years (mean 60.5 years). Fifteen were male and 4 were female (male:female ratio 3.75). The locations in descending order were: lower extremity (7), back (4), scalp (3), upper extremity (2), trunk (2), and face (1). The depth of the SMM ranged from