

PNL2 MELANOCYtic MARKER IN IMMUNOHISTOCHEMICAL EVALUATION OF PRIMARY MUCOSAL MELANOMA OF THE HEAD AND NECK

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Abstract: *Background.* Histologic diagnosis of mucosal melanoma of the head and neck is difficult, requiring immunohistochemical stains which are less reliable than in cutaneous lesions. PNL-2 is a novel marker that has not been examined in mucosal melanoma.

Methods. Nine formalin-fixed tissue sections of mucosal melanoma were stained with PNL-2, human melanoma black (HMB)-45, Melan-A, S-100, and microphthalmia transcription factor (MITF).

Results. Disease in all 9 patients arose from the sinonasal mucosa. Rates of diffuse positive staining with the 4 stains were PNL-2 (77.8%), HMB-45 (77.8%), Melan-A (50%), S-100 (87.5%), and MITF (40%). In 3 patients, PNL2 staining was superior to Melan-A or MITF.

Conclusion. We report the first characterization of PNL-2 staining in head and neck mucosal melanoma. PNL-2 demonstrates high sensitivity for mucosal melanoma, likely superior to Melan-A and MITF, and comparable to HMB-45, with specificity superior to S-100. We advocate inclusion of PNL2 as an important adjunctive marker in the evaluation of these lesions. ©2008 Wiley Periodicals, Inc. *Head Neck* **30**: 771–775, 2008

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While malignant melanoma is a common skin neoplasm encountered in the head and neck, primary mucosal melanoma is rare, accounting for only 4% of melanomas in the head and neck.^{1,2} Nevertheless, the head and neck—particularly, the nasal cavity and sinonasal mucosa—constitutes the most common location of mucosal melanoma.^{3,4}

The appearance of these lesions is highly variable. Clinically, tumors may appear macular, ulcerated, or nodular, with colors ranging from melanotic to violaceous to pink to white. Histologically, cells may be epithelioid, plasmacytoid, or spindled, arranged in sheet-like, alveolar, neurotropic, or desmoplastic configurations. Melanin pigment may be seen in 77% to 90% of lesions.⁵ Occasionally, lesions are amelanotic.

Unlike cutaneous melanoma, the highly variable gross and histologic appearance of mucosal melanoma often creates diagnostic difficulties differentiating between mucosal melanoma and other tumors, such as poorly differentiated carcinoma, lymphoma, plasmacytoma, rhabdomyosarcoma, esthesioneuroblastoma, and others.

Table 1. Patient data.

Patient	Age	Sex	Site	PNL-2	HMB-45	Melan-A	S-100	MITF
1	64	F	Nasopharynx	+	+	+	+	
2	62	M	Left nasal polyp	+	+	+	+	
3	72	M	Left nasal cavity	+	+	Focal	+	
4	91	F	Right anterior ethmoid	+	+	+	+	+
5	66	F	Left ethmoid	+	+	+	+	-
6	59	M	Left maxillary sinus	-	Focal	Focal	+	Focal
7	71	F	Right ethmoid	-	-	-	Focal	-
8	81	F	Left ethmoid	+	+	Focal	+	+
9	74	M	Left nasal cavity	+	+			

Abbreviations: HMB-45, human melanoma black; MITF, microphthalmia transcription factor.
 Note: Staining coded as negative (-), focally positive (Focal), or diffusely positive (+).

Because of these histologic mimics, the diagnosis often depends on immunohistochemistry. The most commonly used melanoma markers in mucosal melanoma include S-100, human melanoma black (HMB)-45, Melan-A, and microphthalmia transcription factor (MITF). However, occasional melanocytic lesions will lack most of these antigens, and rare nonmelanocytic lesions may express some of these melanocyte-related antigens.^{5,6}

In clinical practice, it is not uncommon to use a broad range of antibodies for definitive diagnosis in mucosal melanoma, and there is great interest in additional markers which may offer additional sensitivity and specificity. Increasing surgical use of sentinel lymph node biopsy and frozen section diagnosis underscore the potential value of new, adjunctive markers for melanoma.

PNL-2 is a novel monoclonal antibody, directed against a formalin resistant melanocyte antigen. We compared staining with PNL-2 against HMB-45, Melan-A, S-100, and MITF in mucosal melanoma of the head and neck.

MATERIALS AND METHODS

Patient Selection. Primary malignant mucosal melanoma samples were retrieved from the archival files of the Departments of Pathology, New York University School of Medicine, Mount Sinai School of Medicine (New York, NY) and Shanghai University School of Medicine (Shanghai, China), from 2004 to 2006. IRB approval was obtained. The specimens were surgical biopsies and resections. The clinical history and final pathological diagnostic report was reviewed for each patient.

Immunohistochemistry. Formalin-fixed, paraffin-embedded tissue at 5- μ m serial sections were used for immunohistochemical analysis with appropri-

ate positive and negative controls, using the following antibodies: S-100 (prediluted; Ventana Medical Systems, Tucson, AZ), HMB-45 (prediluted; Ventana Medical Systems), Melan A (1:150; Novocastra, Newcastle-Upon-Tyne, UK), MITF (ABR-Affinity, Golden, CO), and PNL-2 (1:25; DAKO, Glostrup, Denmark). Immunostaining was performed using the Ventana Benchmark XT immunostainer using the manufacturer's deparaffinization, antigen retrieval, and detection reagents. Antigen retrieval consisted of incubating the tissue sections in microwave-heated 0.01M citrate buffer (pH 6.0) and allowing the sections to cool to room temperature before staining. Visualization of immunostaining was by a biotin-avidin immunoperoxidase assay. The sections were counterstained with hematoxylin. Tissue sections with confirmed diagnosis of malignant melanoma were mounted on the selfsame slides as positive controls. As negative controls, tissue sections were incubated with isotype-matched serum without primary antibody. There are no standard criteria for immunostain positivity in malignant melanoma. Similar to other groups,⁷ we interpreted immunostaining based on cell counts as negative, focally positive (<25% of neoplastic cells stained), or diffusely positive (\geq 25% of neoplastic cells stained).

RESULTS

A total of 9 cases were retrieved. Mean age was 71.1 years (range, 59–91 years), with 4 men and 5 women. Disease in all 9 patients arose from the sinonasal mucosa: 4 patients (44%) from the ethmoid sinuses, 3 patients (33%) presented with nasal polyps or masses, 1 (11%) in the nasopharynx, and 1 patient (12.5%) presented with a maxillary sinus lesion. Clinical and staining data are summarized in Table 1.

Table 2. Summary of staining patterns.

	PNL-2	HMB-45	Melan-A	S-100	MITF
Negative, %	22.2	11.1	12.5	0.0	40.0
Positive (focal), %	0.0	11.1	37.5	12.5	20.0
Positive (diffuse), %	77.8	77.8	50.0	87.5	60.0

Abbreviations: HMB-45, human melanoma black; MITF, microphthalmia transcription factor.

Histologically the tumor cells were epithelioid, with pleomorphism and high numbers of mitotic figures. The immunohistochemical profiles are summarized in Table 2, and selected patients are illustrated in Figures 1 and 2. In all patients, the tumor cells were diffusely positive for at least 1 marker. In 1 patient (No. 7), only S-100 was weakly positive (staining <5% of cells).

PNL-2 staining was diffusely positive in 7 of 9 patients (Nos. 1, 2, 3, 4, 5, 8, 9), with no patients having only focal staining. PNL-2 was positive in 1 of 3 patients in whom MITF was focal or negative and 2 of 4 patients in whom Melan-A was focal or negative. PNL-2 staining intensity was stronger than HMB-45. PNL-2 demonstrated excellent specificity, staining only melanocytes.

DISCUSSION

We report the first characterization of PNL2 staining in mucosal melanoma. PNL2 is a novel monoclonal antibody, initially generated against the human SST2 somatostatin receptor. Although PNL2 was ultimately found to not bind SST2, it does bind an unknown antigen on melanocytes and granulocytes.⁷

PNL2 staining of cutaneous melanoma has been preliminarily investigated in 2 reports. In a study of 38 cutaneous melanoma specimens, Busam et al⁷ report positive PNL2 staining in 33 (87%), compared with Melan-A (82%), HMB-45 (76%), tyrosinase (92%), and MITF (82%). PNL2 was not of value for desmoplastic melanoma. As with other melanocyte markers, immunoreactivity to angiomyolipomas was also seen.

Rochaix et al⁸ reported immunostaining of 49 melanoma specimens, in which PNL2 stained 46 (94%), compared with Melan-A (90%) and HMB-45 (90%). In these patients, PNL2 staining was found to be more consistent and strongly positive than HMB-45. Of the 49 specimens, 1 specimen was a mucosal melanoma, which stained positively with PNL2.

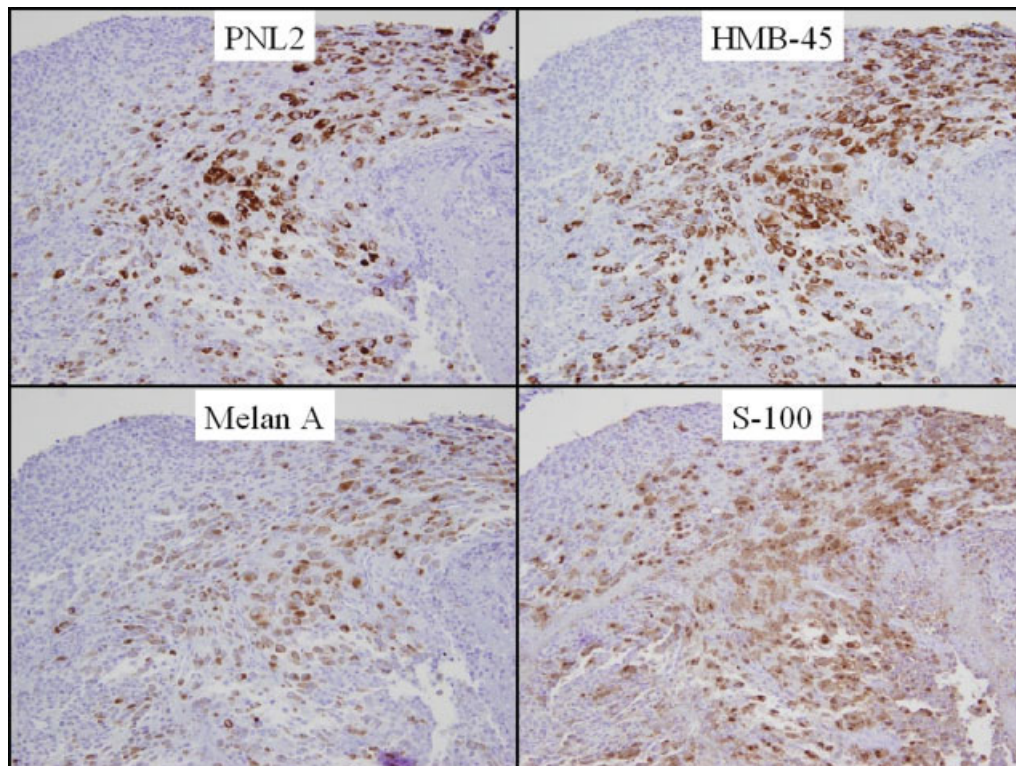


FIGURE 1. Patient 2. Photomicrographs demonstrate high-intensity staining with PNL2 on immunohistochemical analysis. HMB-45, human melanoma black. MITF, microphthalmia transcription factor. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

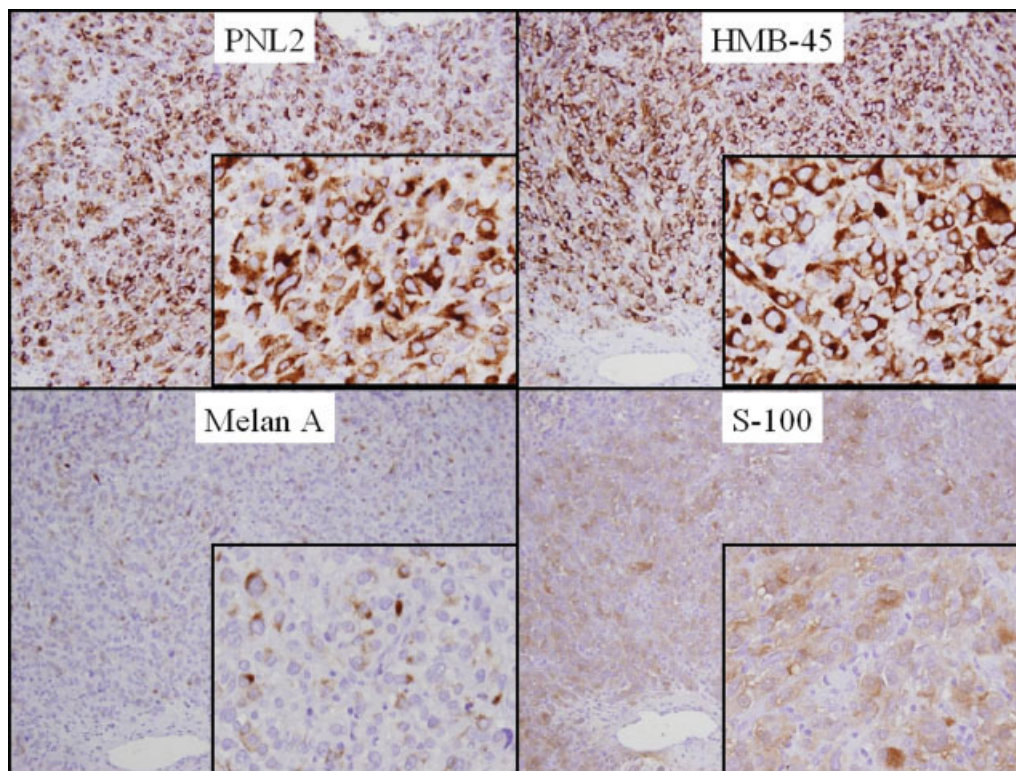


FIGURE 2. Patient 3. Photomicrograph demonstrates diffuse staining with PNL2 on immunohistochemical analysis. HMB-45, human melanoma black. MITF, microphthalmia transcription factor. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Immunohistochemical staining in mucosal melanoma is generally less reliable than in cutaneous melanoma. HMB-45 stains 92% of cutaneous cases, and only 76% of mucosal cases; Melan-A, 95% and 65%; S-100, 88% and 91%; and MITF, 99% and 57%.^{5,6} The reliability of PNL2 immunostaining in mucosal melanoma has not been previously reported.

In our series, PNL-2 demonstrated positive, diffuse staining in 7 of 9 (77.8%) mucosal melanomas. This compared favorably with HMB-45 (77.8%). PNL-2 and HMB-45 positivity were similar, although PNL-2 staining intensity was qualitatively stronger in all patients in whom both stains were positive. This has also been reported by Rochaix et al.⁸ Because PNL-2 and HMB-45 recognize different antigens, differences between these markers may emerge as more specimens are studied.

Sensitivity of PNL2 appeared to be superior to Melan-A (50%) and MITF (40%). In 3 of the 9 patients, PNL2 stained positively when either MITF or Melan-A were negative or only focally positive. Although S-100 showed slightly higher sensitivity (87.5%), S-100 is a nonspecific marker

that also stains adipocytes, chondrocytes, Schwann cells, and myoepithelial cells and is therefore not useful alone when evaluating melanoma.

In our melanoma specimens, PNL-2 demonstrated excellent specificity, staining only melanocytes. Previous reports have also demonstrated that PNL2 has high specificity for melanoma, comparable to HMB-45 and Melan-A, and vastly superior to S-100.⁸ Accordingly, when selecting an immunohistochemical battery for mucosal melanoma, the combination of PNL-2, S-100, and HMB-45 may provide the highest sensitivity and specificity.

CONCLUSION

As expected, the markers in this study carried lower rates of positivity in mucosal melanoma compared with cutaneous counterparts. Employing multiple markers may be helpful if an initial panel of 1 or 2 markers fails to provide a clear positive result. In patients in whom HMB-45 and Melan-A stain focally or ambiguously, the addition of the novel antibody PNL2 may provide addi-

tional diagnostic information. In fact, we advocate the use of PNL-2, S-100, and HMB-45 as a highly sensitive and specific battery of stains for mucosal melanoma, which will likely provide more information than inclusion of either Melan-A or MITF.

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