DIAGNOSIS OF DIFFICULT MELANOCYTIC LESIONS WITH UPDATE ON IMMUNOHISTOCHEMICAL MARKERS

David S. Cassarino, M.D., Ph.D.
Dermatopathology Consultant, Department of Pathology, Los Angeles Medical Center, Southern California Medical Group, and Adjunct Clinical Professor of Dermatology, University of California, Irvine
I have no conflicts of interest to disclose

David S. Cassarino, M.D., Ph.D.
I. INTRODUCTION

➢ Melanoma diagnosis and reporting remains a highly controversial area in dermatopathology, with widely differing approaches based on training and local practices

➢ Ancillary studies (immunohistochemistry and molecular) are being utilized more and more often, especially in more difficult and challenging cases

➢ New markers and molecular studies are being described at a rapid pace

➢ However, it is difficult to determine if they are really useful and cost-effective
Mutations in Melanoma and Nevi

- Numerous recent molecular studies showing mutations associated with various types of nevi and melanoma

- Reported mutations includes:
  - **BRAF** (V600E/K): associated with most nevi and over 50% of melanomas (mostly superficial spreading type)
  - **CDKN2A** (encodes p16): associated with familial melanoma
  - **PTEN**: commonly mutated in melanoma (up to 40% of cases)
  - **NRAS**: associated with melanoma (more in nodular type)
  - **KIT** (encodes c-kit/CD117): mutated in lentigo maligna, acral and mucosal lentiginous melanomas
  - **GNAQ/GNA11**: associated with ocular melanomas and blue nevi
  - **TERT**: telomerase mutations seen in many melanomas (worse pxn)
  - **HRAS**: associated with Spitz nevi and tumors
  - **ALK, ROS1, NTRK1**: rearrangements associated with Spitz tumors
Histologic features, including subtype and prognostic findings (i.e., ulceration, Breslow depth and level of invasion, intensity of lymphocytic infiltrate, and mitotic count) still likely more important in most cases
Many histologic types of melanoma have been described:

- Superficial spreading melanoma
- Lentigo maligna (melanoma in situ) or lentigo maligna melanoma (invasive)
- Nodular melanoma
- Acral lentiginous melanoma
- Mucosal lentiginous melanoma
- Spindle cell/desmoplastic/neurotrophic melanoma
- Nevoid melanoma (“minimal deviation melanoma”)
- Spitzoid melanoma (malignant Spitz tumor)
Rare or Unusual/Controversial types of Melanoma

Malignant CBN (Melanoma arising in or mimicking a CBN)

“Animal type (equine)” melanoma, or possibly “pigmented epithelioid melanocytoma (PEM)”

Myxoid melanoma

Small cell melanoma

Balloon cell melanoma

Signet ring cell melanoma

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<th>Pleomorphic (sarcomatoid) melanoma</th>
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<td>Rhabdoid melanoma</td>
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<td>Melanoma with chondroid or osteoid production</td>
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<td>Primary dermal melanoma</td>
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Rhabdoid melanoma
II. IMMUNOHISTOCHEMISTRY

- Traditional melanocytic markers (S100, Mart-1/Melan-A, HMB45, tyrosinase) **not necessary or useful in most cases**
- Exceptions: spindle cell/desmoplastic melanoma (S100+/SOX10+, KBA62+, Mitf+//-), metastatic melanoma
- In severely atypical/borderline cases, HMB45, Ki-67, and p16 may be useful:
  - Zonation pattern with HMB45 in benign and atypical nevi, not in melanoma:
    - Typically + junctional, weak/negative in the dermis in nevi
    - Stronger dermal staining in most melanomas (but can be negative)
  - Ki-67 typically elevated in melanoma (>15%), neg/low in nevi (<5% of cells)
Immunohistochemistry for p16

- p16 often positive in nevi (> 90%), lost (or only focal/weak) in most melanomas, shows correlation with progression
- Nuclear and/or cytoplasmic staining
  - Nuclear staining may be more specific for nevi
- Studies showing loss of p16 in melanoma compared to conventional, atypical, and Spitz nevi
- Not as well-characterized in severely atypical/borderline Spitz/Reed tumors, epithelioid blue nevi, deep penetrating nevi (DPN), cellular blue nevi (CBN) and atypical/malignant CBN
II. IMMUNOHISTOCHEMISTRY

Newer markers of prognostic/therapeutic importance:

- **BRAF\(^{V600E}\) antibody**: specifically detects mutated BRAF\(^{V600E}\), does not detect V600K or other mutations
  - Highly correlated with molecular studies of BRAF mutation (97% sensitivity, 98% specificity; Long et al., Am. J. Surg. Pathol., Sept. 2012)

- Other markers of possible value include:
  - D2-40, CD31, VEGF (lymphangiogenesis, vasc invasion)
  - CD117 (c-kit): acral & mucosal lentiginous melanoma
  - p16 (loss of expression correlates with worse outcome)
  - BAP1, HRAS, NRAS, ALK, CD43, CD74, cyclin D1, PD1/PDL1
Lymphovascular invasion:
- Poor prognostic factor in many studies
- May use IHC (i.e., CD31 and/or D2-40) to confirm
S100 (red)/D2-40 (brown) double stain
CD117+ mucosal MM. Langer et al. Modern Pathol. 2011;24:495
A distinct subset of Atypical Spitz Tumors is characterized by *BRAF* mutation and loss of BAP1 expression


Abstract

We recently reported that germline mutations in *BAP1* cause a familial tumor syndrome characterized by high penetrance for melanocytic tumors with distinct clinical and histologic features. Melanocytic neoplasms in affected individuals harbored *BRAF* mutations, showed loss of BAP1 expression, and histologically resembled so-called “atypical Spitz tumors” (ASTs). ASTs are an ill-defined and probably heterogenous group of melanocytic tumors that display histologic features seen in both Spitz nevi and melanomas. We hypothesized that a subset of ASTs might harbor genetic alterations seen in the familial tumors. To address this hypothesis, we analyzed 32 sporadic ASTs for *BRAF* mutations and for BAP1 expression. Nine (28%) sporadic ASTs showed loss of BAP1 expression, of which 8 (89%) had concomitant *BRAF* mutations. Only 1 of the BAP1-positive ASTs (4%) had a *BRAF* mutation (P<0.0001). *BRAF*-mutated, BAP1-negative tumors were primarily located in the dermis and were composed entirely or predominantly of epithelioid melanocytes with abundant amphophilic cytoplasm and well-defined cytoplasmic borders. Nuclei were commonly vesicular and exhibited substantial pleomorphism and conspicuous nucleoli. The combination of *BRAF* mutation and loss of nuclear BAP1 expression thus characterizes a subset of ASTs with distinct histologic features. The typical morphology of these tumors and BAP1 immunohistochemistry provide pathologic clues that will enable accurate identification of this subset.
IV. ILLUSTRATIVE CASES
CASE 1

A 45 y.o. white female presented with a forehead papule, slowly enlarging over several years.
CASE 1 DIAGNOSIS: NEVOID ("MINIMAL DEVIATION") MELANOMA
NEVOID MELANOMA

- Nevvoid or “minimal deviation melanoma” (not preferred) is a very difficult and controversial lesion

- Lesion may appear well-circumscribed and symmetric at low magnification

- Have a low index of suspicion for dermal atypia and look for dermal mitotic figures (LEVELS important, Ki-67, p16, and HMB45 may be useful)

- If unsure of diagnosis, get molecular studies and/or send to an expert consultant
CASE 2

35 y.o. male presented with a growing, lightly pigmented papular lesion on the upper arm.
CASE 2 DIAGNOSIS: DESMOPLASTIC SPITZ NEVUS
DESMOPLASTIC SPITZ NEVUS

Desmoplastic (Spitz) nevus features:

▪ Low magnification: symmetric appearance
▪ Usually no or only very small junctional component identified
▪ No (or rare) dermal mitotic figures (not deep or atypical)
▪ No lymphoid aggregates
▪ Nuclei regular, small nucleoli, lack hyperchromasia or prominent atypia
CASE 2

Differential diagnosis:

- Conventional Spitz nevus
- Desmoplastic melanoma (hyperchromatic spindle cells, no epithelioid cells, may have overlying MMIS)
- Blue nevus (pigmented spindled and dendritic cells)
- Dermatofibroma
- Dermatofibrosarcoma protuberans (DFSP)
- Soft tissue tumors, such as leiomyoma and leiomyosarcoma
• Symmetric, well-circumscribed, usually compound proliferation
  - Spindled and epithelioid cells in vertically oriented nests w/streaming (“raining down” appearance)
  - Clefting and Kamino bodies (eosinophilic globules)
SPITZ NEVUS HISTOLOGY:

- Overlying epidermal hyperplasia, hypergranulosis, and hyperkeratosis
- Dermis may show telangiectasia and fibrosis
- Cells have abundant eosinophilic to amphophilic-staining cytoplasm
- Nuclei may be hyperchromatic to vesicular, with prominent nucleoli
- Mitoses may be seen, but should be mostly superficial and not atypical-appearing
- Evidence of maturation with dermal descent
• Diff’nt dx with melanoma can be very difficult, but:
  - Look for combination of spindled and epithelioid cells, maturation with depth, Kamino bodies, mitoses superficial and non-atypical, rare or no apoptotic/necrotic cells
  - Age and clinical history very important (should be smooth, symmetric papule, usually < 40 y.o.)
• Lesions that cannot be definitively diagnosed should be called “atypical Spitz tumor”, or “Spitzoid tumor of uncertain (borderline) malignant potential”
A 4½-year-old child presented with a growing, raised pigmented lesion on her back.
CASE 3

DIAGNOSIS:
- Spitzoid (Nevoid) Melanoma
- Initially misdiagnosed as Spitz nevus by original pathologist
CHILDHOOD MELANOMA

- Rare, but consider melanoma in children when you would in an adults:
  ➔ Features such as cytologic atypia, mitoses, pleomorphism, pagetoid spread, asymmetry
- Melanomas in children can show identical features to those in adults
- Spitzoid and nevoid melanomas can be particularly difficult to identify in this population, as the suspicion for melanoma is often low

6p25 (RREB): normal diploid

9p21 (CDKN2A): deletions

11q13 (CCND1): normal diploid
Melanoma FISH showing multiple chromosomal gains, including amplification of RREB1 (6p25), MYB (6q23), and CCND1 (11q13) probes
CASE 4

Long-standing “mole” with recent enlargement and itching on the cheek of a 56 y.o. white male
CASE 4 DIAGNOSIS: MELANOMA IN SITU MIMICKING A DYSPLASTIC NEVUS
MELANOMA IN SITU MIMICKING A DYSPLASTIC NEVUS

- A significant percentage of melanoma in situ cases, especially lentigo maligna type, can mimic atypical/dysplastic nevi

- Can exhibit bridging, fibroplasia, ascension of single cells along sides of rete ridges, and chronic inflammatory infiltrate

- Clues include asymmetry, poor circumscription, focal confluence, pagetoid spread, hyperchromatic nuclei with prominent nucleoli, and junctional mitoses
LENTIGO MALIGNA MELANOMA MIMICKING A DYSPLASTIC NEVUS

- Kossard et al. described “lentiginous dysplastic nevi of the elderly”, 38% of which showed areas of melanoma in situ (!)

- Farrahi et al. showed that up to 43% of lentigo maligna melanoma in situ, and 25% of invasive lentigo maligna melanoma, showed dysplastic nevus-like features on biopsies

Histologic similarities between lentigo maligna and dysplastic nevus: importance of clinicopathologic distinction

**Background:** Lentigo maligna (LM) can histologically simulate dysplastic nevus (DN). Partial biopsy of LM may lead to misdiagnosis.

**Methods:** One hundred and fourteen cases of LM and LM melanoma (LMM) were diagnosed at the Veterans Affairs Palo Alto Health Care System (1993–2002). Biopsy and excision specimens for 68 in situ and 28 invasive melanomas were classified as having predominant classical LM features, predominant DN-like morphology, or a mixed pattern.

**Results:** Biopsy specimens demonstrated a predominant classical pattern in 38% (25/65) LM and 36% (10/28) LMM, predominant DN-like features in 43% (28/65) LM and 25% (7/28) LMM, and mixed pattern in 15% (10/65) LM and 29% (8/28) LMM. Most LM and LMM biopsies were partial. Significant DN-like features were present in 51% LM and 57% LMM excision specimens. Median age was 72 years for LM and 73 years for LMM, mean lesion diameters were 1.3 and 1.7 cm for LM and LMM, respectively, and 85% of LM and 75% of LMM cases were located on heavily sun-exposed sites.

**Conclusions:** Misdiagnosis of LM or LMM as DN could have devastating results. Large pigmented lesions on sun-damaged skin in elderly individuals should warrant consideration of LM/LMM diagnosis, even in the setting of DN-like features histologically. Excisional biopsy may help to avoid misdiagnosis.

Farinaz Farrahi¹, Barbara M. Egbert¹,² and Susan M. Swetter¹,³

¹Department of Dermatology, Stanford University Medical Center, Stanford, CA
²Pathology and
³Dermatology Services, Veterans Affairs Palo Alto Health Care System, Palo Alto, CA, USA

Susan M. Swetter, MD, Department of Dermatology, Stanford University Medical Center, 900 Blake Wilbur Drive, W0069, Stanford, CA 94305, USA
Tel: +1 650.852.3494
Fax: +1 650.496.2573
e-mail: sswwter@stanford.edu

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Fig. 2. Biopsy of lentigo maligna exhibiting predominant dysplastic nevus-like features. Note the elongated and bridged rete ridges.
LENTIGO MALIGNA MELANOMA MIMICKING A DYSPLASTIC NEVUS

Take home points:

▪ Be very cautious making the diagnosis of atypical/dysplastic nevi in sun-damaged skin

▪ Clinical history and appearance are key for making the correct diagnosis

▪ Recommend complete excision for any atypical lesions with clinically suspicious features
55 year old male with a cystic lesion on the upper back
Necrosis and multiple mitoses
CASE 5
DIAGNOSIS: PRIMARY DERMAL MELANOMA
Primary Dermal Melanoma (PDM)

- **Clinical Criteria:**
  - Solitary nodular or cystic-appearing lesion, usually elderly patients, sun-exposed sites, but no definite site predilection
  - Absence of prior history of invasive melanoma
  - Complete clinical and radiographic exams negative for primary melanoma elsewhere, including mucosal sites, no evidence of regressed lesion clinically
PDM: Histologic and IHC Findings

**Histologic Criteria:**
- Deep dermal/subcutaneous, usually well-circumscribed, tumor with no evidence of epidermal or follicular involvement
- No overlying ulceration or evidence of regression
- No features of malignant blue nevus, animal type melanoma, desmoplastic melanoma, MPNST or clear cell sarcoma

**IHC:** Usually lower Ki67, p53, and cyclin D1 compared to nodular and metastatic melanoma
### Results: Prognostic Findings

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<th>PNM</th>
<th>PDM</th>
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<td>Breslow depth (mm)</td>
<td>-</td>
<td>4.0</td>
<td>8.5</td>
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<td>Median survival</td>
<td>7.5*</td>
<td>84*</td>
<td>94, range 17-124†</td>
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<td>(months)</td>
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<td>5 year survival (%)</td>
<td>6*</td>
<td>67*</td>
<td>92 (est.)</td>
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†2 local recurrences, 1 metastasis and death