BROWN STAINS

MICHAEL A NOWAK, MD
CONFLICTS

• No conflicts with the content of this lecture
IMMUNOHISTOCHEMICAL STAINS

- Antigen-Antibody reaction
- Different techniques
- Different chromogens
- Adjunct to H&E to confirm differentiation
- Diagnostic
- Prognostic
IMMUNOHISTOCHEMICAL STAINS

- Panels or combinations of stains to determine differentiation
- References exist for positive and negative stain percentages for specific lesions
- References exist for percentages of lesions that are positive or negative for specific stains
- Histologic patterns initiate a stain panel
- Some EMRs are equipped with a reflex stain panels
IMMUNOHISTOCHEMICAL STAINS
Specific Patterns

- Pagetoid pattern
- Spitzoid melanocytic pattern
- Epidermal hypermelanosis pattern
DIFFERENTIAL DIAGNOSIS

Erythematous plaque of groin
CLINICAL DIFFERENTIAL DIAGNOSIS

- Eczematous dermatitis
- Psoriasis
- Tinea
- Candidiasis
- Granular parakeratosis
- Malignancy (high index of suspicion)
IMMUNOHISTOCHEMICAL STAINS
Pagetoid Pattern

• Atypical intraepidermal epithelioid cells involving upper and lower layers in small clusters of solitary cells.

• Differential diagnosis: Paget’s disease, extramammary Paget’s disease, melanoma in-situ, pagetoid SCCIS, sebaceous carcinoma, pagetoid reticulosis, merkel cell carcinoma
IMMUNOHISTOCHEMICAL STAINS
Pagetoid Pattern

- Pankeratin
- CK34
- CEA
- CK7
- S100
- Melan A (Mart-1)
- EMA
IMMUNOHISTOCHEMICAL STAINS
Pagetoid Pattern

- Paget’s and extramammary Paget’s disease: CEA and CK7
- Melanoma in-situ: S100, Mart-1 (Melan A), MITF, SOX10
- Pagetoid SCCIS: Pankeratin
- Sebaceous carcinoma: CEA, EMA
- Pagetoid reticulosis: CD3
- Merkel cell carcinoma: CK20 dot-like
CYTOKERATIN 7 (CK7) STAIN: POSITIVE
EXTRAMAMMARY PAGET’S DISEASE
PAGET’S DISEASE

- Mammary
- Extramammary
- Bone
- Microscopic pattern
MAMMARY PAGET’S DISEASE

• 1874 Sir James Paget

• Mammary skin involvement especially the nipple

• Associated with an underlying breast cancer in virtually 100% of cases.

• Poor prognosis.
EXTRAMAMMARY PAGET’S DISEASE

• 1889 Radcliffe Crocker

• Occurs in anatomic sites rich in apocrine glands

• Frequently confined to the skin without an underlying internal cancer.

• Good prognosis.
EXTRAMAMMARY PAGET’S DISEASE

- Sharply demarcated erythematous patch or plaque

- Pruritus and burning pain are common

- Vulva (most common), male genital area, perianal area, and axilla
PERIANAL PAGET’S DISEASE

- Subset of extramammary Paget’s disease frequently not limited to the perianal skin
- Similar to mammary Paget’s in behavior since it is frequently associated with underlying visceral malignancy
- Referrals and bad prognosis
EXTRAMAMMARY PAGET’S DISEASE
Microscopic Findings

• Paget’s cells: Large mucin containing cells

• Single cells or small clusters at all levels of the epidermis

• Basal layer is frequently spared and compressed forming “eyeliner sign”

• Occasional signet ring (vacuolated) cells

Perianal Paget's disease: distinguishing primary and secondary lesions using immunohistochemical studies including gross cystic disease fluid protein-15 and cytokeratin 20 expression.

Nowak MA, Guerriere-Kovach P, Pathen A, Campbell TE, Deppisch LM
Western Reserve Care System
PERIANAL PAGET’S DISEASE

- Primary lesions (limited to skin): CK20 negative/GCDFP-15 positive, good prognosis, high 5 year survival, intraepidermal apocrine carcinoma.

- Secondary lesions (skin and rectal involvement): CK20 positive/GCDFP-15 negative, poor prognosis, low 5 year survival, rectal carcinoma involving skin vs. invasive Paget’s involving rectum.
Signet ring cell perianal paget disease: loss of MUC2 expression and loss of signet ring cell morphology associated with invasive disease.

Grelck KW, Nowak MA, Doval M
PERIANAL PAGET’S DISEASE

- Morphology: Loss of signet ring cell features.
- Immunohistochemical: Loss of MUC2 expression.
- Depth of invasion of greater than 4 mm.
- Associated with a poor prognosis.
<table>
<thead>
<tr>
<th>STAGE</th>
<th>DESCRIPTION</th>
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<tbody>
<tr>
<td>I</td>
<td>Paget’s cells found in perianal epidermis and adnexae without primary carcinoma</td>
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<tr>
<td>IIA</td>
<td>Cutaneous Paget’s disease with associated adnexae carcinoma</td>
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<tr>
<td>IIB</td>
<td>Cutaneous Paget’s disease with associated anorectal carcinoma Paget’s disease in which associated carcinoma has spread to regional nodes</td>
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<tr>
<td>III</td>
<td>Paget’s disease with distant metastases of associated carcinoma</td>
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EXTRAMAMMARY PAGET’S DISEASE

Treatment

- Topical chemotherapy
- Wide local excision (Stage 1 and 2A)
- AP resection (Stage 2B and Stage 3)
- Medical oncology (Stage 4)
- Radiation (Stage 4)
- Referrals
- Long term monitoring
Approach to a patient with extramammary Paget’s disease

![Flowchart Diagram]
EXTRAMAMMARY PAGET’S DISEASE

Summary

• Mammary vs. Extramammary

• Clinical Differential Diagnosis

• Microscopic Differential Diagnosis

• Primary vs. Secondary (GCDFP-15, CK20, MUC2)

• Treatment and referrals

• Long term monitoring
DIFFERENTIAL DIAGNOSIS

Pink papule on scalp
CLINICAL DIFFERENTIAL DIAGNOSIS

- Melanocytic nevus
- Basal cell carcinoma
- Pyogenic granuloma
- Juvenile xanthogranuloma
- Nodular melanoma
IMMUNOHISTOCHEMICAL STAINS
Dermal Spitzoid Melanocytic Pattern

- Dermal epithelioid cells with large nuclei and abundant cytoplasm
- Dermal Spitz nevus, nodular or metastatic melanoma, poorly differentiated squamous cell carcinoma, metastatic carcinoma, anaplastic large cell lymphoma
IMMUNOHISTOCHEMICAL STAINS

Dermal Spitzoid Melanocytic Pattern

- S100
- Melan A (Mart-1)
- SOX10
- HMB-45
- Pankeratin
- LCA
IMMUNOHISTOCHEMICAL STAINS
Dermal Spitzoid Melanocytic Pattern

- Dermal Spitz nevus: S100, Melan A, MITF, SOX10, HMB-45
- Melanoma: S100, Melan A, MITF, SOX10, HMB-45
- Squamous cell carcinoma: Pankeratin
- Metastatic carcinoma: Pankeratin
- Anaplastic large cell lymphoma: LCA
IMMUNOHISTOCHEMICAL STAINS
Dermal Spitzoid Melanocytic Pattern

• Dermal Spitz nevus vs. melanoma
• Additional immunohistochemical stains
• Consultation to an expert for a second opinion
Discordance in the histopathologic diagnosis of melanoma and melanocytic nevi between expert pathologists.

Farmer ER, Gonin R, Hanna MP
Department of Dermatology, Indiana University School of Medicine
IMMUNOHISTOCHEMICAL STAINS
Dermal Spitzoid Melanocytic Pattern

- The combined kappa statistic for the 8 observers and 3 possible outcomes (benign, malignant, or indeterminate) was 0.50.
- 62% had unanimous agreement or only one discordant designation.
- 38% had two or more discordant interpretations.
- The results suggest the criteria for the diagnosis of melanomas and nevi need to be refined and more consistently applied.
- Better stains?
"My guess is that you are about 78, based on the number of food stains on the front of your shirt!"
IMMUNOHISTOCHEMICAL STAINS
Dermal Spitzoid Melanocytic Pattern

- **BRAF** (acquired nevi and superficial spreading melanoma)
- **KIT** (acral and mucosal melanoma and lentigo maligna)
- **HRAS** (subset of Spitz nevi)
- **GNAQ** and **GNA11** (blue nevi and uveal melanoma)
IMMUNOHISTOCHEMICAL STAINS
Dermal Spitzoid Melanocytic Pattern

- BRAF
- p16
- BAP-1
IMMUNOHISTOCHEMICAL STAINS
Dermal Spitzoid Melanocytic Pattern

- BRAF: V600E mutation (VE1 stain) - BRAF gene 7q34
- p16: MTS1 protein - CDKN2A gene 9p21.3
- BAP-1: BRCA-1 associated protein - BAP1 gene 3p21.1
IMMUNOHISTOCHEMICAL STAINS

Stain Results

• BRAF-positive
• P16-positive
• BAP1-negative

• This combination of results = BAPoma
A distinct subset of atypical Spitz tumors is characterized by BRAF mutation and loss of BAP1 expression.

Wiesner T, Murali R, Fried I, Cerroni L, Busam KJ, Kutzner H, Bastain BC
Sloan Kettering, New York, NY
BAPoma

- Wiesner’s nevus
- Clinically indolent similar to Spitz nevus
- Solitary or multiple
- Can be a marker for a germline mutation 3p21.1
BAPoma
Clinical Findings

- Circumscribed pink to tan papule(s) occasionally polypoid
- Average size of 5 millimeters
- Second to third decade of life in any anatomic location
- Solitary or multiple
- Dermoscopy: pink to tan structureless areas and peripheral irregular dots, globules, or network pattern
BAPoma
Histologic Findings

- Dome-shaped dermal epithelioid melanocytic proliferation
- Generally lacks epidermal component
- Frequently biphasic: Large and small melanocytes
- Mild inflammation, minimal pigmentation, and rare mitoses
- Differs from Spitz nevus
BAPoma vs. Spitz Nevus
Histologic Findings

• In contrast to Spitz nevus, BAPoma:
  • Lacks epidermal involvement and hyperplasia
  • Lacks clusters of spindle cells
  • Lacks Kamino bodies
  • BRAF V600E mutation present (positive)
  • BAP-1 loss (negative)
  • Like Spitz nevus p16 expression is preserved (positive)
BAPoma

What would I see on a report?

- Atypical Spitzoid tumor with BAP-1 loss
- BAP-1 inactivated Spitzoid nevus (‘BAPoma’)
- BRAF V600E positive
- BAP1 negative
- P16 cyclin-dependent kinase inhibitor 2 (CDKN2A gene) positive
RESIDUAL NEVUS, R/O NEUROFIBROMA

COMBINED MELANOCYTIC NEVUS, INCLUDING A COMPONENT OF DESMOPLASTIC BAP-1 INACTIVATED SPITZOID NEVUS (‘BAPOMA’) (D48.5) (RIGHT LATERAL NECK)

NOTE: Two different specimens have been evaluated jointly as the basis for this consultation, and I understand that the second biopsy represents persistence at the site of the first biopsy. For both specimens, my differential diagnosis included a combined desmoplastic Spitz nevus on one hand and a combined melanocytic nevus with a component of BAP-1 inactivated spitzoid nevus (‘BAPoma’) on the other. Immunostaining for BAP-1 demonstrates loss of nuclear positivity in the large melanocytes that are present, thereby corroborating that BAP-1 genomic loss has occurred in those cells. Expression of p16 is preserved, and thus concomitant CDKN2A genomic loss has not occurred. The labeling combination discussed in the two preceding sentences is confirmatory of the diagnosis of ‘BAPoma’.

In summary, the combination of results derived from conventional microscopy coupled with immunohistochemistry is confirmatory of the diagnosis of BAP-1 inactivated spitzoid nevus (‘BAPoma’). A key issue in the evaluation of a patient with a ‘BAPoma’ is the determination regarding whether the proliferation stems from a sporadic somatic mutation or whether instead the proliferation is a harbinger of an underlying germline mutation. Most combined ‘BAPomas’ stem from somatic mutations, but the overall clinical context must be used to make this distinction.
BAPoma

- Continuum 1
  - BAPoma (p16+) $\rightarrow$ Intermediate $\rightarrow$ Melanoma with BAP1 loss (p16-)

- Continuum 2
  - Spitz nevus (p16+) $\rightarrow$ Intermediate $\rightarrow$ Spitzoid melanoma (p16-)
BAPoma
What do I do next?

- Complete excision for solitary lesions
- Look for additional lesions
- Inquire about eye tumors and mesothelioma
- Long term surveillance similar to a melanoma patient
- Referrals for multiple lesions (germline mutation)
- Multiple lesions followed for change in clinical appearance, radiologic studies, and genetic counseling
BAPoma
Germline Mutation

- Associated with multiple BAPoma lesions
- Frequently microscopically homogeneous
- Familial mesothelioma (non-asbestos related)
- Uveal melanoma
- Cutaneous melanoma
- Other malignancies especially renal cell carcinoma
**GERM-LINE MUTATIONS**

- **Germ-line mutation**
- **Parental Gametes**
- **Embryo**
- **Organism**
- **Gametes of Offspring**

**SOMATIC MUTATIONS**

- **Somatic mutation**
- **Patch of affected area**
- **None of gametes carry mutation**
BAPoma
Summary

• Wiesner’s nevus

• Different from Spitz nevus

• BAP-1 inactivated Spitzoid nevus (‘BAPoma’)

• Solitary (excise) or multiple (follow and referrals)

• Germline mutation is associated with multiple nevi, uveal melanoma, cutaneous melanoma, familial mesothelioma, and other cancers
ROADS?

WHERE WE'RE GOING,
WE DON'T NEED ROADS.
DIFFERENTIAL DIAGNOSIS

Tan-brown patch on sun exposed skin
CLINICAL DIFFERENTIAL DIAGNOSIS

• Solar lentigo
• Seborrheic keratosis
• Pigmented actinic keratosis
• Lichenoid keratosis
• Lentigo maligna
IMMUNOHISTOCHEMICAL STAINS

Epidermal Hypermelanosis Pattern

- Epidermal hypermelanosis with a gradient of low to high density of melanocytes and minimal cytological atypia
- Solar lentigo, pigmented actinic keratosis, lichenoid keratosis, lentigo maligna
IMMUNOHISTOCHEMICAL STAINS
Epidermal Hypermelanosis Pattern

- Pankeratin
- S100
- Melan A (Mart-1)
- HMB-45
- MITF
- SOX-10
IMMUNOHISTOCHEMICAL STAINS
Epidermal Hypermelanosis Pattern

- Solar lentigo: Pankeratin positive
- Pigmented actinic keratosis: Pankeratin positive
- Lichenoid keratosis: Pankeratin positive
- Lentigo maligna:
  - Pankeratin negative (silhouette of unstained melanocytes)
  - S100, Melan A, HMB-45, MITF, SOX10 positive
IMMUNOHISTOCHEMICAL STAINS
Lentigo Maligna

- Sufficient sampling is key: Multiple shaves (not punch)
- High density: Confluence of melanocytes in basal layer
- Follicular involvement
- Dermal-epidermal and intercellular separation
- Moderate involvement of spinous layer
- Multinucleate melanocytes and minimal atypia
The American Journal of dermatopathology 2013 Jun;36(2)

Diagnostic Utility and Comparative Immunohistochemical Analysis of MITF-1 and SOX10 to Distinguish Melanoma In Situ and Actinic Keratosis.

Buonaccorsi JD, Prieto VG, Torres-Cabala CA, Suster, S
Department of Pathology, University of Texas - M.D. Anderson Cancer Center
PRAME Expression in Melanocytic Tumors.

Lezcano C, Jungbluth AA, Nehal KS, Hollman TJ, Busam KJ
Sloan Kettering, New York, NY
PRAME
PReferentially expressed Antigen in MElanoma

- 400 melanocytic tumors including 155 primary and 100 metastatic melanomas and 145 melanocytic nevi.

- Diffuse nuclear immunoreactivity for PRAME was found in 87% of metastatic and 83.2% of primary melanomas.

- Among melanoma subtypes, PRAME was diffusely expressed in 94.4% of acral melanomas, 92.5% of superficial spreading melanomas, 90% of nodular melanomas, 88.6% of lentigo maligna melanomas, and 35% of desmoplastic melanomas.

- PRAME expression was seen in both in situ and nondesmoplastic invasive melanoma components.
PRAME
PReferentially expressed Antigen in MEIanoma

- 86.4% of the 140 cutaneous melanocytic nevi were completely negative.

- Occasional melanocytes were positive in 13.6% of cutaneous nevi.

- Rare junctional melanocytes were also positive in solar lentigines and benign nonlesional skin.

- Useful to support a diagnosis of melanoma.

- Margin assessment of a known PRAME-positive melanoma.

- Expression in nevi, solar lentigines, and benign nonlesional skin can represent a pitfall
CLINICAL DATA: A) JUNCTIONAL MELANOCYTIC NEVUS VS. GROWING NEVUS VS. SPITZ NEVUS, RULE OUT ATYPICAL MELANOCYTIC PROCESS. A FEW PERIPHERAL DOTS SEEN ON DERMOSCOPY AND SMALL BROWN MACULE, BIT LARGER IN THE LAST MONTH. LESION SIZE: 1 MM; 2 MM PUNCH PERFORMED.

DIAGNOSIS: JUNCTIONAL MELANOCYTIC NEVUS WITH PIGMENTED EPITHELIOID MELANOCYTES, EXCISED IN PLANES OF SECTION EXAMINED (D22.22) (LEFT CENTRAL POSTAURICULAR)

NOTE: I have examined multiple level sections and a dual stain for PRAME/Melan-A. This Melan-A component of this stain highlights the architecture of this lesion and the PRAME component is negative. Often nuclear staining for PRAME is noted in melanoma in situ and melanoma, a finding not observed in this case.
Unstable solar lentigo: A defined separate lesion.

Byrom L, Barksdale S, Weedon D, Muir J
Queensland, Australia
Unstable Solar Lentigo

- A lentigo with areas of melanocytic hyperplasia not extending past the margin of the lesion.
- Macular pigmented lesion arising on sun-damaged skin.
- Clinically differ from usual solar lentigines, often being solitary or larger and darker than adjacent solar lentigines.
- They can arise in close proximity to lentigo maligna
IMMUNOHISTOCHEMICAL STAINS
Unstable Solar Lentigo

• Single lesions can demonstrate changes of solar lentigo, unstable solar lentigo, and lentigo maligna.

• Unstable lentigo is likely a precursor to lentigo maligna.

• Lentigo maligna can arise within a solar lentigo through an intermediate lesion (unstable solar lentigo).

• Difficulties in the diagnosis of single cell predominant melanocytic proliferations

• Unstable lentigo is now recognized as a separate entity.
IMMUNOHISTOCHEMICAL STAINS

Junctional Melanocytic Proliferation

- Continuum
  - Solar lentigo → Unstable lentigo → Lentigo maligna
EPIDERMAL HYPERMELANOSIS PATTERN

Summary

- Sufficient sampling is key
- Density, confluence, and follicular involvement
- Nuclear stains are superior (MITF and SOX10)
- PRAME
- Lentigo maligna: Complete excision
- Unstable lentigo: Follow and additional sampling
IMMUNOHISTOCHEMICAL STAINS
Notable Stains

- CK7
- BAP-1
- p16
- PRAME
- SOX10
IMMUNOHISTOCHEMICAL STAINS
Notable Stains

• CK7: Positive in Extramammary Paget’s disease
• BAP-1: Negative in BAPOMA
• p16: Positive in nevi and negative in melanoma
• PRAME: Positive in melanoma and negative in nevi
• SOX10: Nuclear melanocytic marker like MITF that does not overemphasize melanocyte density
Summary

- Why do we do immunohistochemical stains?
  - Identify or confirm differentiation
  - Panels are used because of overlapping stain results
  - Histologic patterns initiate brown stains
  - Concepts change after new stains are available
“Dang. I bet that stains.”