Atypical Melanocytic Proliferations

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Conflicts of Interest

- Chairman Scientific Advisory Board – Caliber I.D. Inc.
- Member Scientific Advisory Board – MELA Sciences Inc.
- Consultant – Novartis
- Consultant – Alnylam
Top of the lesion

Maturation:
Reduction in nest size and cell size as base is approached

Bottom of the lesion
Classical Spitz nevus: clinical morphology
- characteristically on the face of a child
- recent onset and rapid growth
- dome-shaped papule or nodule
- pink-tan or reddish color
  - becomes brown with diascopy
- epiluminescence microscopy:
  - large globules in light tan background
  - radial streaming in a starburst array

Spitz nevus
- Darier and Civatte (1910): noted that some pigmented childhood lesions were indolent
- Spitz (1948): defined giant cells as predictive of benign outcome in “juvenile melanoma”
- McGovern (1967): coined the term “Spitz nevus”
- other appellations:
  - spindle cell nevus: (Helwig, 1954)
  - epithelioid cell nevus: (Kerman and Ackerman, 1960)
  - spindle and/or epithelioid cell nevus (Paniago-Periera and Maize, 1978)

Spitz nevus: histology
- compound Spitz nevi: 65%
- junctional Spitz nevi: 10%
- dermal Spitz nevi: 25%
  - mainly seen in adults

Spitz nevus: histomorphology
- Architecture:
  - sharply circumscribed dermoepidermal melanocytic proliferation
  - an inverted cone with base parallel to epidermis and apex in reticular dermis
  - large junctional theques separated by cleft-like spaces from hyperplastic epidermis
  - “raining-down” vertical spindled fasicles

Spitz Nevus: Histology
- More common childhood features (especially in first 2 years of life):
  - edema
  - telangiectasia
  - epithelioid cell predominance
  - papillomatosis
  - abrupt maturation – ie – one or two layers at base – common in infancy
CLASSIC SPITZ’S NEVUS: Nest-epidermal dyshesion with cell to cell cohesion

Spitz nevus: cytomorphology

3 cell types:

- ganglion cell
- epithelioid melanocytes:
  - large smooth-contoured nuclei with prominent nucleoli, evenly-distributed chromatin, chromatinic rims of uniform thickness; low N/C ratio
  - abundant eosinophilic cytoplasm; spherical shape
- spindled melanocytes:
  - similar nuclei but fusiform clear to variably pigmented cytoplasms
THE CHARACTERISTIC CYTOMORPHOLOGY OF THE SPITZ'S NEVUS

Spitz nevus: histomorphology

- **cytology:** maturation phenomenon
  - nest and cell sizes diminish towards depth of biopsy, where nests break up into single cells with an infiltrative pattern
  - morphometry confirms diminishing nuclear sizes (Steiner et al., 1994; Bergman et al., 1996)
  - careful 40X magnification to assess for even scattered small nuclei at base is warranted
  - 500 cubic microns vs 775 in melanoma
  - maturation absent in some cases

- **mitotic figures**
  - present in 20% of cases
  - superficial and junctional areas
  - marginal mitoses (ie, within 0.25 mm of lesional edge) prompt concern (McCarthy 1994)
  - >5 per mm² should prompt concern

- **intravascular proliferations**
  - seen in 14% of childhood Spitz nevi (Howatt and Variend, 1985)

Spitz nevus: Pagetoid spread

- Present to some degree in most cases
  - prominence in children>adults and in acral>other sites
  - most prominent centrally near maximal nested junctional component
  - does not extend at lateral edges past nested component
  - single cell and nested pattern
  - may involve eccrine/follicular adnexae

Prominent Pagetoid infiltration with Epithelioid Cytology:
- Confined mainly to center of lesion

**Practice point:**
- The epithelioid cytology is more common in childhood but is unusual in adults and may point to a melanoma when present
Pagetoid spread in this Spitzoid lesion in a 43 year old was a clue to melanoma

Spitz nevus: histomorphology

- Kamino bodies: (Kamino et al., 1979)
  - seen in 60% of all types of Spitz nevi
  - eosinophilic hyalin bodies 30-40 microns
    - PAS-D-positive/trichrome-positive
    - bundles of filaments and basement membrane components derive from either keratinocyte or melanocyte cytosolic shell
  - coalescence/smaller size variably held to suggest melanoma (Weedon, 1984) or benignancy (McCarthy et al., 1994)

Classic Spitz’s nevus

Kamino Bodies:
- extracellular filament bundles
- trichrome and PAS +
Practice point: Kamma bodies melanoma and melanocytic dysplasia are smaller and more confluent than those in Spitz nevi

Variants of The Spitz’s Nevus

Plaque-like Spitz nevus

- Most commonly on thighs of women from 20 to 40 years of age.
- Clinically a plaque up to 1.0 cm in size.
- Color variable, pink, brown to black, or flesh colored.
- Clinical diagnosis usually nevus,? Atypia; if pink or flesh colored, lichenoid dermatitis or flat wart, respectively.

Plaque-like Spitz nevus

- Normal to slightly hyperplastic epidermis
- Prominent intraepidermal nests with sometimes often pagetoid spread.
- Single and small nested melanocytes in dermis of same morphology as in epidermis- mitoses rare.
- Dermal fibrosis common with prominent vessels.
- Minimal melanophages.

Plaque type Spitz Nevus: Regressive stromal changes and banal Spitzoid melanocytes
Pagetoid Spitz nevus

Busam and Barnhill (1995)

- clinical features:
  - pigmented macule < 0.4 cm in young adult
- histopathology:
  - single cell > nested Pagetoid array of epithelioid cells showing sharp circumscription
  - cells lack angular nuclei
  - abundant cytoplasm with uniform melanization
  - no dominant dermal nodule

Pagets spread phenomenon

- Melanoma
- Carcinoma
  - Paget’s disease
  - Bowen’s disease
  - Sebaceous carcinoma
  - Merkel cell carcinoma

Frequency of Pagetoid Melanocytosis

<table>
<thead>
<tr>
<th>Condition</th>
<th>Percent</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma</td>
<td>96</td>
<td>25</td>
</tr>
<tr>
<td>Spitz Nevus</td>
<td>38</td>
<td>47</td>
</tr>
<tr>
<td>Nerves of palms and soles</td>
<td>61</td>
<td>18</td>
</tr>
<tr>
<td>Pigmented spindle cell nerves</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Recurrent nevus</td>
<td>60</td>
<td>10</td>
</tr>
<tr>
<td>Vulvar nevus</td>
<td>80</td>
<td>5</td>
</tr>
<tr>
<td>Nevus of infancy and childhood</td>
<td>100*</td>
<td>3</td>
</tr>
<tr>
<td>Ordinarily acquired nevus</td>
<td>none</td>
<td>3</td>
</tr>
</tbody>
</table>

*cases predicted for pagetoid melanocytosis

Desmoplastic Spitz nevus

- Clinical features (Reed et al; 1975):
  - presents in adult life as tan or flesh colored nodule <1.0cm
  - extremities, trunk; duration >3 years
  - spares palms and soles
- differential diagnosis:
  - scar, dermatofibroma, appendage tumor, or desmoplastic melanoma

Desmoplastic Spitz nevus

- Differential diagnosis:
  - other forms of sclerosing nevus, ie:
    - desmoplastic type A nevus
    - desmoplastic combined nevus
    - sclerosing fibrohistiocytic lesion
    - desmoplastic melanoma

Spindle cell nevus

(Reed et al., 1975)

- Clinical features:
  - black or dark dome shaped lesion
  - 2-6 mm in diameter
  - located on proximal extremities or trunk
  - classically young woman (second decade)
  - preferentially on knees + elbows in children
  - 50% on thigh or arm in 1 series (Sagebiel, 1984)
Spindle cell nevus
(Reed et al., 1975)

- Histological features: architecture
  - a superficial plaque-like growth involving epidermis+/-dermis (2/3 compound)
  - vertically oriented spindled cells in retia; horizontal disposition when fused
  - fine papillary dermal collagen present; lamellar fibroplasia usually absent
  - Pagetoid spread common; whole nests classic
  - Inflammation frequent but regression rare

Practice point: spindle cells are uniform in size and shape with uniform nucleolation; spindle cell melanomas show nuclear overlay with higher N/C ratios + pleomorphism
Dysplastic variant of a Spitz's nevus

- There are atypical nevomelanocytic proliferations which manifest overlap features between a Spitz's nevus and a dysplastic nevus.
- The cardinal features are a cytomorphology defining that encountered in the Spitz's nevus in concert with an architecture typical for a dysplastic nevus.
- **The designation:** Dysplastic nevus with overlap cytomorphologic features with a Spitz's nevus/dysplastic Spitz's nevus

Spindle / Epithelioid Cell Nevus

- Typical
- Atypical
Atypical Spitz Nevus

- Superficial expansile nodules
- Asymmetry
- Impaired maturation
- Rare dermal mitosis, especially deep in adults

- These are changes that, as single features, would prompt the diagnosis of "Atypical Spitz Nevus"
BAPoma: Historical Perspective

- Weisner et al. first described 2 families that had multiple skin lesions varying from a few to 50 lesions.
- Found to show autosomal dominant inheritance.
- Families had a history of uveal melanoma as well as cutaneous melanomas.
- Genetic studies revealed germ line mutations in the BAP1 (BRCA1-associated protein 1), a tumor suppressor gene located on chromosome 3 (3p21).
- Ubiquitin carboxy-terminal hydrolase, functions as a deubiquitylating enzyme for protein substrates.
- It was then found that spontaneous cases also occur without germ line mutations.

Other names include:

- NEMMP: highly atypical nevoid melanoma-like melanocytic proliferations
- MBAIT: melanocytic BAP1-mutated atypical intradermal tumors
- BAPoma
- Also likely misclassified in the literature as
  - epithelioid atypical Spitz tumors
  - melanoma
- However, these are molecularly distinct and behave non-aggressively

BAPoma: Clinical Findings

- A family history of cutaneous and/or ocular melanoma
- Orange to red, semitranslucent, papular, or pedunculated lesions, often < 1 cm
- Lesions are usually firm, often surrounded by a halo of pigmentation
- Sun exposed areas are most commonly involved including head, neck, arms, and lower extremities but lesions can occur anywhere
- Studies have shown the BAP1 mutation to occur in melanomas, renal cell carcinoma, meningioma, and other cancers
- A recent study has shown that basal cell carcinomas in the familial setting show the mutation and may be used for screening

Skin lesions: Inconspicuous, skin-colored, dome-shaped papules

Image Provided by Thomas Weisner
**BAPoma: Histology**
- Lesions are predominantly dermal but occasionally exhibit junctional nests
- The lesion presents as an expansile nodule that often is associated with a peripheral benign dermal nevus
- There is a spectrum of prominent epithelioid cells with ample cytoplasm and well defined borders to cells with small cytoplasm and small hyperchromatic nuclei with a nevoid appearance
- Many cells resemble Spitz nevus cells but with marked nuclear pleomorphism and hyperchromasia. There is also a clear nucleolus with condensed chromatin and a prominent nucleus.
- Mitoses are infrequent

**Family History**

- Ocular melanoma
- Dermal nevi
- 7 cutaneous melanomas (4 nevoid)
- 1 cutaneous melanoma
- Dermal nevi
- BAP-1 negative melanocytic proliferations

**Mutational Screening**

- Germline BAP1 mutation: p.Asp236Glyfs*7 found as carrier status for the three affected members of this family
- Tumor BAP1 biallelic inactivation: (from 7 tumors: 1 SSM, 1 nevoid melanoma, 5 atypical nevomelanocytic proliferations)
  - p.Asp236Glyfs*7, a LOH in the nevoid melanoma
  - p.Ser123Lysfs*3, a separate somatic BAP1 mutation in a nevomelanocytic proliferation

**Results**

**BAP1**
- BAP1, or BRCA1-associated protein 1/ubiquitin carboxy-terminal hydrolase, functions as a deubiquitylating enzyme for protein substrates
- BAP1 germline mutations have been seen in familial cancers such as mesotheliomas and meningiomas
- Hereditary studies among ocular and cutaneous melanoma kindreds with germline BAP1 mutations have helped identify and molecularly characterize these ubiquitous yet banal acting epithelioid cell melanocytic tumors

**Role of the Pathologist**
- The pathologist expertise is critical in the discovery and documentation of the Bap1 lesion because of the genetic implication. This association of so many tumor with the mutation has now been included Online Mendelian Inheritance in Man (OMIM) database, (#614327).
BAPoma

- The finding of this gene is very significant especially in light of the fact that it may occur spontaneously and apparently predisposes to other malignancies.

- Screening for the lesion may be performed by immunohistochemical studies, Sanger sequencing, or screening of basal cell carcinomas or possibly other benign tumors in affected patients.

Atypical Spitz’s tumor

- Subset of Spitzoid melanocytic proliferations with a worrisome histology but indeterminate biologic behaviour
  - architecture resembles VGP melanoma
  - cytology resembles conventional Spitz
  - metastases, when present, tend to confine to regional lymph nodes
  - often larger than usual Spitz nevus: >2cm
  - clinical appearances otherwise similar to common Spitz’s nevi

Atypical Spitz’s tumor

- Histomorphology divided by a scoring system into low/intermediate and high risk
- Conclusion of study: the only independent prognostic variables were:
  - age > 10 years
  - ulceration
  - involvement of subcutis
  - mitotic rate >6 per square mm
Atypical Spitz’s tumor
(Busam and Barnhill, 1995)

- **Classical Spitz**
  - Size: <1 cm
  - symmetrical
  - sharp demarcation
  - regular nesting
  - absent deep extent
  - absent expansile nodule

- **Atypical Spitz**
  - Size: >1 cm
  - asymmetrical
  - sharp demarcation-ve
  - irregular nesting
  - deep extension
  - expansile nodule present
Criteria to Distinguish Spitz Nevus From Malignant Melanoma

Table derived from:
Spitz Nevus versus Spitzoid Malignant Melanoma: An Evaluation Of the Current Distinguishing Histopathologic Criteria
Walsh N, Crotty K, Palmer A, McCarthy S. Human Pathol 29: 1105-1112

Malignant

- Breslow thickness (thicker than 2.0mm)
- Diameter greater than 1.0 cm
- Asymmetrical
- Marked pagetoid spread, especially in teenagers, of epithelioid cells
- Ulceration (childhood)
- Dermal nests larger than intraepidermal nests
- Marked nuclear hyperchromasia
- Dermal mitosis > 5 mm² (childhood)

Malignant (cont)

- Mitosis in papillary dermis ≥4 mm²
- Atypical mitosis
- Marginal mitosis
- Large pigment granules especially in deep nests
- Large distinctly more pleomorphic deep dermal nests
## Benign

- Symmetry
- < 1.0cm in diameter
- Sharp circumscription of epidermal components
- Epidermal hyperplasia
- Nests relatively uniform in size and shape
- Small uniform nests toward base

## Benign (con’t)

- Single cells at base
- Cells uniform from side to side
- Predominance of spindle cells
- Rare or no mitosis in lower third
- No mitosis at base
- Maturation (diffuse)
- No regression

## Spitzoid Melanoma

- The classic Spitzoid melanoma is seen mainly in the pediatric population most commonly in the head and neck
- The differential diagnosis is primarily the high risk atypical Spitz tumor
- The consensus is that biological behavior is unpredictable
- Longer term follow up may reveal a clinical course no different from other types of melanoma

## Spitzoid Melanoma

- **Architecture**: Dominantly dermal based expansile nodule with variable permeation of the subcutis
- Numerous bizarre appearing giant cells similar to those described in the Spitz nevus but with greater pleomorphism and striking nuclear atypia; the cells assume a confluent sheet like disposition.
**Treatment**

- **Spitz nevi and variants**: complete excision with minimal morbidity
- **Atypical Spitz’s tumors**: excision with current melanoma margins. Narrow margins would be inadequate; sentinel node biopsy with high risk lesions greater than 1 mm.
- **Spitzoid melanoma**: conventional melanoma therapy with sentinel node biopsy for lesions greater than 1 mm.
Childhood Melanoma

Sites of involvement: Head and neck. Especially scalp when arising in congenital nevi

Dorsal surface favored for lesions arising in congenital nevi

For all types: Head and neck > extremities > trunk > generalized skin
Case:

• 13 year old boy with a “bump” on the scalp for some time that suddenly grew rapidly and ulcerated.
• Clinical Diagnosis: Atypical nevus Vs. Melanoma Vs. Other
• Died within 2 years
Case:

- 13 years-old, female.
- Primary tumor was 2.2 mm thick (Clark level IV, ulcerated, with a mitotic rate of 3 mit./mm²) and located on the foot. Her lymph node was positive at the time of excision.
- Died of metastatic melanoma 2 years later.
Atypical Spindle/Epithelioid Cell Nevus Resembling Childhood Melanoma

Clues for the diagnosis of Melanoma

Pleomorphism from one area to another at some level
Deep expansile nodule with monomorphic cells
Multiple deep and marginal atypical mitosis
Lack of maturation with individual cell necrosis
Fine pigment in deep cells

Childhood Melanoma Survival

All patients with metastatic melanoma arising in giant nevi dead within 5 years in study of Trozak et al in 1974

Patients with melanoma arising de novo or in other lesions, including small congenital nevi had 34% survival at 5 years according to Melnick et al

Survival of congenital melanoma poor, >40% dead within 18 months

Spitz nevi versus melanoma


- 102 Spitz nevi studied for 11p copy number increases by FISH; 11p is site of hRAS gene
- 11.8% had at least 3X copy number ↑
- Tumors with 11p copy number larger, more often dermal with desmoplasia, characteristic cytology and an invasive growth pattern
- Sequence analysis of hRAS showed oncogenic mutations in 67% of cases with 11p copy number ↑ vs only 5% of tumors with no copy number ↑
Spitz nevus vs melanoma

- Comparative genomic hybridization of 17 Spitz nevi versus melanoma
- 13 Spitz nevi had no chromosomal anomalies
- 3 Spitz nevi had gains of 11p
- 1 Spitz nevus had a gain of 7q21
- Melanomas had deletions of 9p (92%), 10q (63%), 6q (28%), + 8p (22%); gains of chromosomes 7 (50%), 8 (34%), 6p (28%), 1q (25%)

Molecular Diagnosis in Nevi and Melanoma

- Fluorescent In Situ Hybridization (FISH)
- Comparative Genomic Hybridization (CGH)

Fluorescence in Situ Hybridization

- Identifies chromosomal copy number aberrations
- Fish probes (short DNA fragments)
- Slide containing 5µm thick paraffin embedded section of tumor (test sample)

Fish probes

- **RREB1, 6p25**: Ras responsive element binding protein 1
- **CEN6**: Centromere 6
- **MYB, 6q23**: v-myb myeloblastosis viral oncogene homologue
- **CCND1, 11q13**: cyclin D1

Gerami & Zembowicz, Arch Pathol Lab Med, 2011
# 4 Fish Criteria for Melanoma diagnosis

1. More than 38% of enumerated cells contain >2 signals for CCND1, or
2. More than 55% of nuclei contain more signals for 6p25 than for centromere 6, or
3. More than 40% of nuclei contain fewer signals for MYB than for centromere 6, or
4. More than 29% of cells have >2 RREB1 signals.

# FISH

- These probes and the diagnostic criteria were developed based on the CGH data of Bastian et al at UCSF
- Validation studies were performed at Northwestern
- Analysis of 86 nevi and 83 melanomas rendered a sensitivity of 86.7% and a specificity of 95.4%

## FISH in ambiguous melanocytic lesions

**Gaiser et al, Modern Pathol;2010**

- FISH/Clinical Behavior: 60% sensitivity, 50% specificity

## Improved FISH

- 322 tumors, including 152 melanomas and 170 nevi
- A more discriminatory probe set: 9p21, 6p25, 11q13, and 8q24
- Sensitivity of 94%
- Specificity of 98%

## Risk Assessment for Atypical Melanocytic Neoplasm

- 75 Atypical Spitz tumors (US & Aus.)
- 64: benign behavior (5 year f/u)
- 11 with metastasis and/or death (3)
- Greater risk: homozygous 9p21 deletion
- High risk: 6p25 or 11q13 gains

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**A Highly Specific and Discriminatory FISH Assay for Distinguishing Between Benign and Malignant</br>Melanocytic Neoplasms**

Pedram Gerami, MD,*w Gu Li, PhD,z Pedram Pouryazdanparast, MD,* Beth Blondin, BS,z Beth BellRuss, BS,* Carl Slank, MSc,z Jing Du, MSc,z Joan Guitart, MD,*w Susan Jewell, PhD,z and Katerina Pestova, PhD, MBAz

*Am J Surg Pathol 2012;36:808–817*
Risk Assessment for Atypical Melanocytic Neoplasm

- 6 cases with isolated 6q23 loss showed no evidence of met or death (96 month f/u)
- 64 cases with benign behavior: 23.4% has a positive FISH result


Comparative Genomic Hybridization (CGH)

- Chromosomal CGH: when the test and normal DNA is hybridized to metaphase chromosomes
- Array CGH: hybridization to DNA microarrays
- CGH represents the first efficient approach to scanning the entire genome for variations in DNA copy numbers

Comparative Genomic Hybridization (CGH)

- 96% of 132 melanomas had chromosomal copy number aberrations
  - Gains in 6p, 1q, 7q, 8q, 17q, 11q, and 20q
  - Losses in 9p, 9q, 10p, 10q, and 6q
- 13% of all nevi evaluated (54) showed the same gain in 11p ► Spitz nevi

FISH and CGH in ambiguous melanocytic lesions

Gaiser et al, Modern Pathol;2010
- Fish results compared with CGH and long-term clinical follow up in 22 melanocytic proliferations, 12 of which were ambiguous lesions.
- FISH/Clinical Behavior ► 60% sensitivity
- 50% specificity
- CGH/Clinical Behavior ► Lesions that metastasized showed significantly more chromosomal aberrations

Molecular tests in Pigmented Lesions: Conclusion

“Correlation between precise molecular attributes and exacting histomorphology is in its infancy”

Tim McCalmont et al
J Cutan Pathol, 2011
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