Update on the Clinico-Pathological and Molecular Diagnosis of Melanocytic Lesions

Belfast pathology
Arnaud de la Fouchardière MD, PhD
Lyon, France

Conflicts of interest
None to declare

What is new? Today’s Menu

• Realm of «Molecular Pathology»
• New concepts
• New techniques applied to FFPE
• New entities

What is new?

• Realm of «Molecular Pathology»

What is new?

• Realm of «Molecular Pathology»
• New concepts

Step by step histo-molecular progression from nevus to melanoma

The Genetic Evolution of Melanoma from Precancer Lesions
In many lesions there is a progressive gain of molecular anomalies linked with incremental microscopic atypia.

The «gray zone» of diagnosis is visible

<table>
<thead>
<tr>
<th>Benign</th>
<th>Atypical</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>ĀNevus</td>
<td>Atypical nevus</td>
<td>Melanoma</td>
</tr>
<tr>
<td>ĀBlue nevus</td>
<td>Atypical blue nevus</td>
<td>Melanoma ex-blue nevus</td>
</tr>
<tr>
<td>ĀSpitz Nevus</td>
<td>Atypical Spitz tumor</td>
<td>Spitzoid Melanoma</td>
</tr>
<tr>
<td>ĀDPN</td>
<td>Atypical DPN</td>
<td>Plexiform Melanoma</td>
</tr>
</tbody>
</table>

Wiesner et al. pathology 2015
What is new?

- Realm of «Molecular Pathology»

- New concepts
  Step by step histo-molecular progression from nevus to melanoma
  « Integrative analysis »

« Integrative analysis »
From concepts to tools

- Melanocytic tumors represent a wide variety of lesions.

Clinical features

- Location
- Age of appearance
- Size
- Type of sun exposure/Phototype
- Recent modifications:
  - Color
  - Shape
  - Volume/ulceration
  - Pain/itching

Melanoledge project
Integrative analysis

- Embryogenesis
- Clinical features
- Microscopy/morphology
- Immunophenotype
- Genomic profile
- Mutation status
- Clinical evolution

« Integrative analysis »
From concepts to tools

- Melanocytic tumors represent a wide variety of lesions.
- There are many ways of viewing differences.
- The goal is to combine as many «viewpoints» as possible in order to ultimately visualize melanocytic lesions in a more accurate manner.

MD: Giant congenital nevus
M15 Back (Dr de Carrère): BAP1-inactivated melanocytic tumor

F34 Abdomen (Dr Bellili): Superficial Spreading Melanoma

F6 arm (picture Dr Dadban): Spitzoid melanoma

Microscopy/morphology
- Low magnification (Silhouette)
- High magnification (cytology)

Molecular Pathology of melanocytic tumors

Mutations
- « Driver »
- BRAF
- NRAS
- NF1
- HRAS
- CKIT
- GNAQ
- GNA11
- …
- « Oncogenetic »
- CDKN2A
- CDK4
- MiTF
- BAP1
- …
- « Passenger »
- PTEN
- hTERT
- p53
- …
Main drivers

- BRAF V600E
- GNAQ/11 exon 4 or 5
- NRAS exon 2 or 3
- BRAF V600K
- Kit exon 11, 13, 17 or 18

Molecular Tools: use is guided by clinical morphological features

- IHC
- FISH
- NGS
- CNV

What is new?

- Realm of «Molecular Pathology»
- New concepts
- New techniques applied to FFPE

Antibodies, Next Generation Sequencing, RNA-seq
Immunohistochemistry

It’s all about choosing the adapted antibody(ies) for the selected task(s)

Antibodies

- Melanocytic antibodies
  - MelanA
  - S100
  - HMB45
  - PNL2
  - MiTF
  - SOX10
  - ...

- Anomaly-specific antibodies
  - ALK
  - RO1
  - NTRK1
  - MET
  - P16
  - BAP1
  - PDL1
  - ...

- Other antibodies
  - D2-40
  - CD68
  - ...

Specificity vs Sensitivity

HMB45

Why perform IHC?

- Confirm melanocytic lineage
- Visualize the melanocytes
- Benign vs Malignant
- Molecular characterization

IHC to confirm melanocytic lineage

- Unpigmented dermal or ulcerated tumor (No recognizable junctional melanocytes)
- Unpigmented metastases
- Desmoplastic melanoma
IHC to confirm melanocytic lineage

- Unpigmented dermal or ulcerated tumor (No recognizable junctional melanocytes)
- Unpigmented metastases
- If usual melanocytic markers are negative
- Combination of SOX10 and MiTF nuclear positivity favors melanocytic origin
MiTF

IHC for Molecular characterization
- Point mutations
- Gene fusions
- Loss of function (tumor suppressor genes)
  « Theragnostic » tools

Point mutations
- BRAF V600E
- NRAS Q61R

BRAF VE1

BRAF VE1

NRAS Q61R

Melanoma arising from a large congenital nevus
Kinase Fusions IHC Screening tool

- ALK
- ROS1
- NTRK1
- Pan-NTRK
- MET

Currently no reliable BRAF or NTRK3 fusion Ab

Spitzoid tumors with kinase fusions

Spitzoid tumors with ALK fusions

IHC Gene Fusions

Spitzoid tumors with kinase fusions

Lesional spectrum of low grade tumors
- Kinase fusions allow a subclassification of tumors with (but not limited to) Spitzoid morphology
- Genotype/morphotype link? Work in progress
- Potential therapeutic targets
- Potential prognostic factors (BRAF fusion worse?)

Drivers are mutually exclusive Nevoid Vs Spitzoid?

BRAF V600E mutation vs Kinase fusions
« Raise your hand » vote

BRAF mutation  Fusion
BRAF VE1 Ab

IHC to explore loss of gene function (tumor suppressor genes)

- BAP1
- p53
- ...

Usefulness of molecular pathology in cutaneous melanocytic tumors

**Level 1**
100%

- Morphological analysis with knowledge of essential clinical information: age of patient, size, localization and evolution of the tumor (recent modification for instance)

**Level 2**
10%

- IHC screening if «benign vs malignant» doubt: 4 Ab panel (anti-MelanA, HMB45, anti-p16, Ki67) with a red chromogene for thick lesions (>1 mm).

**Level 3**
5%

- Complementary IHC study searching for a specific anomaly guided by morphology and 4 Ab panel (anti-BAP1, anti-ALK, etc.).

**Level 4**
1%

- Extensive molecular analysis in the event of a wide surgery (digital amputation, etc.), in pediatric variants of melanoma or rare melanocytic tumors (unclassified)
- Case specific combination of techniques:
  - FISH: 4 color CDKN2A (P16), fusions specific probes (ALK, ROS1, etc.)
  - Gene sequencing accessing somatic mutations (BRAF, NRAS, GNAQ, GNA11, HRAS, etc.)
  - CGH-array; RNA-seq analysis.
Hyperbole of uncertainty/technique

Clinical evolution
- Local relapse
- Nodal extension
- Metastatic dissemination
- Site
- Biological comparison to primary tumor

Melanoledge project
Convergence of viewpoints
- Embryogenesis
- Clinical features
- Microscopy/morphology
- Immunophenotype
- Genomic profile
- Mutation status
- Clinical evolution

What is new?
- Realm of «Molecular Pathology»
- New concepts
- New techniques applied to FFPE
- New entities

BAP1-inactivated melanocytic tumors (BAPimt)
Arnaud de la Fouchardière, MD, PhD
Lyon, France

- Kinase fusions in spitzoid tumors
- Melanocytic tumors with loss of BAP1 expression
**BAP1**: BRCA1- Associated Protein 1
A gene with multiple faces

Loss of nuclear BAP1 expression = loss of gene function

Loss of BAP1 expression in melanocytic lesions of the skin
Distinct scenarii

**Somatic > germline** mutation of BAP1
- Children or young adults
- Female > Male
- Sun-exposed areas
- Modification of a nevus
- Growing unpigmented nodule < 1 cm
- Inflammatory features
- Multiple lesions: germline

**BAPIHC**
Normal staining

Compound nevus

Loss of BAP1 expression
in melanocytic lesions of the skin

Solitary BAPImt
- BAPImts(s)/ melanoma(s) in the context of a BAP1 cancer predisposition syndrome (germline mutation)
- Sporadic epidermal-linked melanomas (DM)
- Melanomas arising from/mimicking cellular blue nevus

BAPImts

F, 14 back
M, 56 Arm
BAPimts

F25 multiple lesions since age 11

BAPimts keep the morphologic features of the nevi they arose from

M 27 with uveal melanoma
Congenital-like nevus with nodule

Lipidic inclusions

Lipidic inclusions
F 8: Ear; familial history of uveal melanoma

Lateral combined aspect

M, 15 Back

F, 19 Back
Nests of nevoid cells

Large epithelioid and nevoid melanocytes +/- lymphocytes
Large epithelioid and nevoid melanocytes +/- lymphocytes

Clonal large unpigmented melanocytes

Clonal large unpigmented melanocytes

Multinucleations
BAP1 IHC

BRAF V600E mutation association (> 80%)

BRAF mutation IHC (VE1)

BAPimts
- No formal guidelines
- Unknown prognosis
- Complete surgical removal
- Follow-up
- Identify patients needing oncogenetic counseling

Tumors associated with a BAP1 germline mutation
- Atypical cutaneous melanocytic tumors (BAPimts)
- Uveal melanoma
- Cutaneous melanoma
- Leptomeningeal melanoma
- Mesothelioma (pleural and abdominal)
- Clear cell renal cancer
- Meningiomas
- Multiple basal cell carcinomas
- These cancers arise at a younger age
- Maybe other cancers: lung, stomach, pancreas, CholangioK
- Probably incomplete cancer spectrum: ongoing work

Frequency of germline mutations?
Estimated a <16% frequency of germline mutation

We recommend, following the diagnosis of a Melanocytic tumor with loss of BAP1 expression, performing a BAP1 immunohistochemistry in all other cutaneous melanocytic tumors removed previously or simultaneously and all skin melanomas.

Malignant transformation of BAPimts?

M43, history in childhood of BAPimt on the neck
3 cm buttocks mass that grew in a few months

Malignant transformation of BAPimts?

M43, history in childhood of BAPimt on the neck
3 cm buttocks mass that grew in a few months

Malignant transformation of BAPimts?

M43, history in childhood of BAPimt on the neck
3 cm buttocks mass that grew in a few months

Malignant transformation of BAPimts?

M43, history in childhood of BAPimt on the neck
3 cm buttocks mass that grew in a few months

BAP1
Malignant transformation of BAPimts?

Differential Diagnosis
- Spitz nevus
- Nevoid melanoma
- Dystrophic nevus
- BAPimt-like melanocytic tumors

DD: Spitz nevus
21/07/2017

**Nevoid melanoma ex-nevus**

*Image: M, 64 Neck, BAP1*

**Dystrophic nevus**

*Image: BAP1*

**« BAPimt-like » lesions**

*Image: F29, shoulder, BAP1*

**BAP1 take home messages**

- BAPology is a fascinating new science
- BAP1-inactivated melanocytic tumors can identify carriers of a germline syndrome with cancer-predisposition.
- Isolated cases with somatic mutations prevail.
- Exophytic combined architecture
- Large unpigmented epithelioid and nevoid dermal melanocytes +/- lymphocytes.
- IHC is an excellent technique to identify loss of function.

**Global Take home messages**

- Extremely active field for molecular pathology
- Molecular techniques are strongly dependant on high quality clinical and morphological analysis
- Enhancing classifications improves patient care
## Definition of Primary Tumor (T) - AJCC 8th Edition

<table>
<thead>
<tr>
<th>T Category</th>
<th>Thickness</th>
<th>Ulceration status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tis (melanoma in situ)</td>
<td>Not applicable</td>
<td>Not applicable</td>
</tr>
<tr>
<td>T1</td>
<td>≤1.0 mm</td>
<td>Unknown or unspecified</td>
</tr>
<tr>
<td></td>
<td>&lt;0.8 mm</td>
<td>With ulceration</td>
</tr>
<tr>
<td>T1b</td>
<td>&gt;0.8 mm</td>
<td>Without ulceration</td>
</tr>
<tr>
<td></td>
<td>0.8–1.0 mm</td>
<td>With or without ulceration</td>
</tr>
<tr>
<td>T2</td>
<td>&gt;1.0–2.0 mm</td>
<td>Unknown or unspecified</td>
</tr>
<tr>
<td>T2a</td>
<td>&gt;1.0–2.0 mm</td>
<td>Without ulceration</td>
</tr>
<tr>
<td></td>
<td>&gt;2.0–4.0 mm</td>
<td>With ulceration</td>
</tr>
<tr>
<td>T3</td>
<td>≥2.0–4.0 mm</td>
<td>Unknown or unspecified</td>
</tr>
<tr>
<td>T3a</td>
<td>≥2.0–4.0 mm</td>
<td>Without ulceration</td>
</tr>
<tr>
<td></td>
<td>&gt;4.0 mm</td>
<td>With ulceration</td>
</tr>
<tr>
<td>T4</td>
<td>&gt;4.0 mm</td>
<td>Unknown or unspecified</td>
</tr>
<tr>
<td>T4a</td>
<td>&gt;4.0 mm</td>
<td>Without ulceration</td>
</tr>
<tr>
<td>T4b</td>
<td>&gt;4.0 mm</td>
<td>With ulceration</td>
</tr>
</tbody>
</table>