89 Emerging Molecular Diagnostic Tests and Therapies for Melanoma

Aleodor Andea MD

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33 W. Monroe, Ste. 1600
Chicago, IL 60603
Malignant melanoma is the leading cause of death among cutaneous neoplasms. The diagnosis and differentiation of melanoma from benign nevi is currently based on morphology however; in a significant number of cases a definitive diagnosis of melanoma is not possible. Recent molecular studies have revealed genomic differences between melanomas which harbor numerous chromosomal gains and losses and benign nevi which have no detectable chromosomal aberrations. Assays evaluating these abnormalities are ready to be implemented into clinical practice and could become important tools in the diagnosis of this deadly disease. On the therapy side, agents targeting specific pathways active in melanomas are being aggressively investigated with some, including BRAF inhibitors, getting ready for primetime. The session will focus on the utility of comparative genomic hybridization using metaphase chromosomes and microarrays as well as fluorescent in situ-hybridization in establishing a diagnosis of melanoma. In addition, data reflecting the efficacy of the newly FDA approved BRAF inhibitor (vemurafenib) in metastatic melanoma will be presented and the role of BRAF mutation testing discussed.

- Recognize the categories of melanocytic lesions for which an accurate histologic diagnosis is difficult.
- Determine appropriate ancillary studies that may help establish a correct diagnosis.
- Become familiar with new agents used for the treatment of melanoma.

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Aleodor Andea MD
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Molecular Pathology
Molecular Pathology
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Emerging Molecular Diagnostic Tests and Therapies for Melanoma

Aleodor Andea, MD
Associate Professor of Pathology and Dermatology
Director of Dermatopathology Section
University of Alabama at Birmingham
Birmingham, USA
aandea@uab.edu

Disclosure information

I have no financial or industrial affiliation to disclose.

Aleodor A Andea

Overview

1. Problems in the diagnosis
2. Molecular alterations (with implications for diagnosis or treatment)
3. Diagnostic assays
4. New therapies
Melanoma diagnosis

<table>
<thead>
<tr>
<th>NEVI</th>
<th>MELANOMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small</td>
<td>Large</td>
</tr>
<tr>
<td>Symmetric</td>
<td>Asymmetric</td>
</tr>
<tr>
<td>Well-circumscribed</td>
<td>Circumscription</td>
</tr>
<tr>
<td>Predominantly nested</td>
<td>Nested architecture</td>
</tr>
<tr>
<td>Uniform</td>
<td>Nest uniformity</td>
</tr>
<tr>
<td>No</td>
<td>Confluent growth at DEJ</td>
</tr>
<tr>
<td>Present</td>
<td>Maturation</td>
</tr>
<tr>
<td>Absent</td>
<td>Cytologic atypia</td>
</tr>
<tr>
<td>Absent</td>
<td>Mitoses in dermis</td>
</tr>
<tr>
<td>Absent</td>
<td>Atypical mitoses</td>
</tr>
</tbody>
</table>

- ~1-2 million bx/year in US to rule out melanoma
- ~3%-6% of bx are melanomas
- In most cases dx can be made on histopathology
- 2.3%-25% of cases with diagnostic discrepancies
  

Reasons for discrepancy

- Ambiguous lesions with overlapping criteria
  - Atypical Spitz nevi
  - Atypical blue nevi
  - Recurrent melanocytic nevi
  - Nevi in acral, genital or mammary line regions
  - Nevus sebaceous
- Inter-observer variability
Need for a better test

- Prevent under/overtreatment of patients
- Reduce medical costs associated with unnecessary treatment
- Impact positively on patient care
- Most common reason for medical malpractice in pathology


Molecular alterations in melanocytic neoplasms

- MAP kinase alterations
- Replicative senescence in melanocytic lesions

GROWTH FACTORS

RAS

RAF

Y

TOPLASM

H-Ras, K-Ras, N-Ras
A-Raf, B-Raf, C-Raf1

MAP Kinase

MEK

ERK

PROLIFERATION

NUCLEUS

MEK-1, MEK-2
ERK-1, ERK-2
GROWTH FACTORS

MAP Kinase

KIT mutations in 17% of melanomas

Curtin et al., N Engl J Med, 2005

MAP Kinase pathway activation in melanocytic lesions

30% of melanomas

Albino et al., Oncogene 1989

MAP Kinase pathway activation in melanocytic lesions

Spitz's nevi

Bastian B et al. Am J Pathol 2000
GROWTH FACTORS

RAS B-RAF YTOPLASM

MAP Kinase pathway activation in melanocytic lesions

40-60% of melanomas, 80% of nevi

CYTOPLASM

NUCLEUS


B-Raf mutations in melanocytic lesions

<table>
<thead>
<tr>
<th>Lesion</th>
<th>% with B-Raf mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanomas</td>
<td>50-80%</td>
</tr>
<tr>
<td>Nevus</td>
<td></td>
</tr>
<tr>
<td>Congenital</td>
<td>86%</td>
</tr>
<tr>
<td>Intradermal</td>
<td>88%</td>
</tr>
<tr>
<td>Compound</td>
<td>70%</td>
</tr>
<tr>
<td>Clark's</td>
<td>52-80%</td>
</tr>
<tr>
<td>Spitz</td>
<td>0%</td>
</tr>
<tr>
<td>Spitzoid Melanomas</td>
<td>0%</td>
</tr>
</tbody>
</table>

Replicative senescence in melanocytic lesions

- Chromosomes are protected by telomeres and the enzyme telomerase

Greider and Blackburn EH, Nature 1989
Szostak et al, Cell 1989
Molecular tests differentiating melanoma from nevi

- IHC
- Expression microarrays
- CGH
- FISH
Ki-67

Nevus (<5%)

Melanoma (>5%)

Ki-67 problems

Spindle cell melanoma

Indeterminate lesion

HMB-45/ Cyclin D1

Nevus

Melanoma
HMB-45 problems

Combined Nevus  Melanoma


p16

Spitz Nevus  Melanoma

Positive in 100% of Spitz nevi

Negative in 32-50% of melanomas


Expression microarray
• 120 melanocytic lesions
• FFPE
• Combimatrix CustomArray Platform
• Hierarchical clustering

Problems with tissue microarrays

• Expensive
• Require microdissection
• Difficult to reproduce results

Multi-marker assays
Multi-marker assays

Gene expression microarrays → Small set of discriminant markers → IHC

- ARPC2
- FN1
- RGS1
- SPP1
- WNT2

Kashani-Sabet et al. PNAS 2009, Haqq et al. PNAS 2005

IHC

Top: 0, 1+, 2+, 3+
Bottom: 0, 1+, 2+, 3+

Algorithm for discrimination
95% specificity
91% sensitivity

Kashani-Sabet et al. PNAS 2009
Comparative Genomic Hybridization

- Screens the entire genome for gains and losses in DNA material in one experiment

- Variants:
  - Conventional CGH
  - Array based CGH

Conventional CGH

The ratio of green:red signals along each chromosome is determined
Array CGH

- Arrays of genomic bacterial artificial chromosome (BAC) clones

Classifying Melanocytic Tumors Based on DNA Copy Number Changes

Bastian B et al., Am J Pathol 2003

CGH in Melanocytic lesions

- 54 benign nevi
  - 27 Spitz nevi
  - 19 Blue nevi
  - 7 Congenital nevi

- 132 MM
  - 22 Acral location
  - 108 non-Acral

Bastian B et al., Am J Pathol 2003
Chromosomal Aberrations in Ambiguous Melanocytic Lesions

- Spitz nevi:
  - no abnormalities
  - gains on 11p (12-18%)
- Congenital nevi: no abnormalities
- Cellular blue nevi: no abnormalities

Conclusion

Potential diagnostic test for ambiguous melanocytic lesions.
Disadvantages of CGH

- Requires 30-50% pure tumor cells
- Does not allow histologic correlation
- Cannot detect tumor subpopulations

FISH

Fluorescence In Situ Hybridization (FISH) as an Ancillary Diagnostic Tool in the Diagnosis of Melanoma

Pallavi G. Grover, MD*; Susan S. Jesun, PhD**; Larry E. Merriam, PhD***

Beth Blonder, BS**; John Glade, BS**; Patricia Raffait, BS**; Paul Munchak, FT, MLS**

Elisa Zipser, BS**; Jennifer Currier, BS**; Leticia M. Martinez, MLS; Scott R. Harned, MCC

Susan Charnas, MS; Nicholas A. Kadrocy, BS**; Juan Escobar, MD*

Tang H. Lawhorne, MPh; Susan Bower, MPh*

Philip G. Levitt, MBA**; and Warren C. Bartner, MPh*
• Training cohort 301 melanocytic tumors
  – 148 melanomas
  – 153 nevi

• Validation cohort
  – Unequivocal lesions:
    • 83 melanomas
    • 86 nevi
  – 27 ambiguous cases with clinical follow-up
    • 6 cases developed metastases
    • 21 free of disease at > 5 years follow-up

• Validation cohort:
  – 72 of 83 melanomas FISH positive
    • 87% sensitivity
  – 82 of 86 nevi FISH negative
    • 95% specificity
    • 4 nevi were FISH positive


• 27 cases with indeterminate histology
  – 6 developed metastases, all FISH positive
    • 100% sensitivity
  – 21 disease free at > 5 years, 15 FISH negative
    • 71% specificity


Rapid Communication
Diagnosis of cutaneous melanocytic tumours by four-colour fluorescence in situ hybridisation

Advances in Skin Res. 2009. Vol. 10, No. 6, 515-517

20 Nevi 20 Melanomas
19/20 negative 18/20 positive
Specificity 95% Sensitivity 90%
Sensitivity of Fluorescence In Situ Hybridization for Melanoma Diagnosis Using RREB1, MYB, Cep6, and 11q13 Probes in Melanoma Subtypes

<table>
<thead>
<tr>
<th></th>
<th>Superficial Spreading N=70</th>
<th>Lentigo Maligna N=28</th>
<th>Nodular N=22</th>
<th>Acral lentiginous N=3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>81%</td>
<td>82%</td>
<td>91%</td>
<td>100%</td>
</tr>
<tr>
<td>Most common alter 6p25 gain (RREB1)</td>
<td>73%</td>
<td>68%</td>
<td>82%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Classification ambiguous melanocytic lesions with FISH and correlation with clinical long-term follow up

- 22 total lesions
  - Sensitivity: 60%
  - Specificity: 33%

- 12 ambiguous lesions

  - FISH worked in 8 cases
    - 5 malignant (3 FISH positive)
    - 3 benign (1 FISH negative)

Other potential applications

- "Nevoid" melanomas vs "Mitotically active" nevi
Mitotically active nevus
N=10

10/10 cases FISH negative

Nevoid melanoma
N=10

10/10 cases FISH positive


Other potential applications

• “Nevoid” melanomas vs “Mitotically active” nevi
• Intranodal nevus vs Metastatic Melanoma

<table>
<thead>
<tr>
<th>Metastatic melanoma</th>
<th>Nodal nevus</th>
</tr>
</thead>
<tbody>
<tr>
<td>FISH status</td>
<td></td>
</tr>
<tr>
<td>20 cases FISH positive</td>
<td>16 cases FISH negative</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>Specificity</td>
</tr>
<tr>
<td>83%</td>
<td>94%</td>
</tr>
</tbody>
</table>

Other potential applications

- “Nevoid” melanomas vs “Mitotically active” nevi
- Intranodal nevus vs Metastatic Melanoma
- Blue nevus vs Blue nevus-like metastatic melanoma

<table>
<thead>
<tr>
<th></th>
<th>Blue nevus-like metastatic melanoma</th>
<th>Blue nevus</th>
</tr>
</thead>
<tbody>
<tr>
<td>FISH status</td>
<td>9 cases FISH positive</td>
<td>10 cases FISH negative</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>90%</td>
<td>Specificity 100%</td>
</tr>
</tbody>
</table>

6p25 gains

Other potential applications

- “Nevoid” melanomas vs “Mitotically active” nevi
- Intranodal nevus vs Metastatic Melanoma
- Blue nevus vs Blue nevus-like metastatic melanoma
- Micro-staging in melanomas with associated nevi
Conclusion

• FISH provide additional criteria to help diagnose histologically ambiguous cases
• This test should be performed in conjunction with standard histopathologic evaluation

How to use molecular testing

• Not in all cases!
• Only when histology is ambiguous
• Clinically relevant
• Trust your clinical judgment

Is this final?

• Sensitivity not great
• Interpretation is labor intensive
• Technically challenging
• Expensive
• More probes/cutoff points need to be evaluated
Melanoma treatment

FDA approved therapy
- High-dose interleukin-2
- Dacarbazine
- Response rates: 7-20%
- No improvement in survival

New FDA approved drugs
- Ipilimumab (YERVOY)
  - FDA approval: 3/25/2011
- Vemurafenib (ZELBORAF)
  - FDA approval: 8/17/2011
Iplimumab

- Blocks cytotoxic T-lymphocyte–associated antigen 4 (CTLA4)
Ipilimumab phase 3 trial

Hodi et al. N Eng J Med, 2010,

Ipilimumab phase 3 trial

Robert et al. N Eng J Med, 2011,
Vemurafenib

- BRAF V600E inhibitor

MAP Kinase pathway activation in melanocytic lesions

40-60% of melanomas, 90% is V600E
PLX4032 (Vemurafenib)

- **Phase I:**
  - Complete or partial tumor regression 81%

- **Phase II (BRAF Inhibitor in 2 (BRIM2)):**
  - Single-arm study
  - 132 patients
  - 2% complete response
  - 53% show a >30% tumor reduction
  - 30% stable disease

Flaherty et al. N Engl J Med, 2010,

PLX4032 (Vemurafenib)

- **Phase III (BRIM3):**
  - 675 patients
  - Vemurafenib vs Dacarbazine
  - 63% reduction in risk of death
  - 74% reduction in risk of death and disease progression

Vemurafenib phase 3 trial
Side effects

• Rash
• Photosensitivity
• Hair loss
• Joint pain
• Liver problems
• Arrhythmias
• Allergic reactions
• Cutaneous SCC (26%)

Cobas 4800 BRAF V600 Mutation Test

• FDA approved companion diagnostic test
• Detects BRAF V600E mutation
• Real time PCR

References

Molecular alterations in melanoma

References
CGH/ FISH in melanoma

References

Vemurafenib

Other