Molecular Aspects of Melanocytic Neoplasia

Iwei Yeh MD, PhD
University of California, San Francisco

Melanocytic Nevi Arise From Initiating Oncogenic Mutations

Normal melanocyte → Nevus → Melanoma

Initiating Oncogenes in Common Nevii

- NRAS
- BRAF
- Unknown

Thanks to:
Boris Bastian
Timothy McCalmont
Philip LeBoit
Swapna Vemula
Jeff North
Laura Pincus
Beth Ruben
Thaddeus Mully

Pollock et al. Nature Genetics 2003
Initiating Oncogenes in Blue Nevi

VanRaamsdonk et al. 2010 NEJM

Initiating Oncogenes in Spitz Tumors

Bastian et al. 2000 Am J Pathology

HRAS Spitz Nevus
Initiating Oncogenes in Spitz Tumors

- BRAF + BAP1 loss
- HRAS
- Unknown

Initiating Oncogenes in Spitz Tumors

- unknown
- RET Fusions
- ALK Fusions
- NTRK1 Fusions
- HRAS
- BRAF/NRAS +BAP1
- ROS1 Fusions
- BRAF Fusions

Wiesner et al. 2014 Nature Communications
Cancer arises from accumulating alterations in the genome

**Initiating Oncogenes as Targets for Therapy**

<table>
<thead>
<tr>
<th>Oncogenic Event</th>
<th>FDA approved in MM</th>
<th>Clinical Trials in MM</th>
<th>FDA approved in cancer</th>
<th>Clinical Trials in cancer</th>
<th>In vitro</th>
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<tbody>
<tr>
<td>BRAF mutation</td>
<td>Vemurafenib; dabrafenib+trametinib</td>
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<td>NRAS mutation</td>
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<td>KIT mutation</td>
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<td>ALK fusion</td>
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<td>RET fusion</td>
<td>cabozantinib, vandetinib</td>
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<td>ROS1 fusion</td>
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<td>NTRK1 fusion</td>
<td>AZ23</td>
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</table>
Cancer arises from accumulating alterations in the genome

Detecting copy number abnormalities:

- FISH (fluorescence in situ hybridization)
- CGH (comparative genomic hybridization)

Melanomas Frequently Demonstrate Copy Number Aberrations

FISH: What gets analyzed?

- RREB1 (Ras-responsive element binding protein 1) on chromosome 6p (6p25)
- CEP6 at the centromere of chromosome 6
- MYB (myeloblastosis viral oncogene) on chromosome 6q (6q23)
- CCDN1 (cyclin-D1) on chromosome 11q (11q13)
- 9p21 (CDKN2A), 8q
**FISH: Potential effectiveness in melanoma diagnosis (Gerami)**

- 86.7% sensitivity
- 95.4% specificity
- (Mixed validation cohort of 301 tumors with known behavior; often thick melanomas)

Gerami et al. American Journal of Surgical Pathology 2009

**FISH: effectiveness in the context of Spitzoid melanoma**

- 70% sensitivity
- Can be improved with assessment for 9p21 homozygous loss

Gammon et al. American Journal of Surgical Pathology 2012
What gets FISHed?

- Spitz vs. spitzoid MM
- Combined nevus vs. MM ex nevus
- Acral nevus vs. acral MM
- Dysplastic nevus vs. nevoid MM
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A FISH-negative tumor

- Is it not melanoma?
- Is it a melanoma that lacks aberrations RREB1, MYB, or CCND1 (is it a tumor with no copy number abnormality within chromosomes 6 and 11)?

FISH advantages

- Potentially applicable to single cell
- Quick turnaround (within a week)
- Easily adaptable to existing equipment, including microscopes, hybridizers, etc.
**FISH limitations**

- Operating in a darkfield environment, tumor cells may be overlooked
- 4-6 probes are commonly utilized
- Only chromosomes 6 and 11 were analyzed in the initial protocol
- With many probes, technical costs can become prohibitive

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**Comparative Genomic Hybridization (CGH)**

Chromosome CGH provides "cytogenetic" resolution ~ 10 Mb

Kallioniemi et al. Science 1992
Array CGH

Our array CGH platform
Agilent 4x180k human array

Snijders et al., Nat. Genet. 1998
CGH

CGH loss

CGH gain

CGH loss
Gain of 11p

Classification of Copy Number Changes

By degree
- Loss: 1 copy loss
- Homozygous Loss
- Gain: increase of 1-3 copies
- Amplification: increase of > 3 copies

By size
- Focal
- Chromosome Arm
- Whole chromosome

By timing
- Germline
- Somatic
CGH by arrays

- Analysis of DNA fragments in comparison to an array library of 180,000 normal genomic DNA segments
- Interpretation by software (Agilent/Nexus)
- Graphical tabulation of genomic gain/loss

What gets analyzed by CGH?

- Spitz vs. spitzoid MM
- Combined nevus vs. MM ex nevus
- Acral nevus vs. acral MM
- Dysplastic nevus vs. nevoid MM
- Cellular blue nevus vs. blue-like MM
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36 year old woman
Spitz vs. melanoma
Not melanoma; it’s a desmoplastic Spitz
NOT a Spitz, it’s melanoma

**Advantages of aCGH**
- Entire genome is examined
- Timely (2 week turnaround)
- Expense similar to FISH

**Limitations of aCGH**
- Inapplicable to single cell analysis
- Significance of small genomic anomalies, such as small monoaberrations, remain undefined
- Thickness threshold ~0.5 mm
56 year old man
Mole vs. melanoma
Nevoid melanoma, not a deep penetrating nevus

60 year old male; back

Indication: diagnostic uncertainty (probably triggered by spitzoid melanocytes in an older patient)
Combined nevus w/ BAP-1 loss
BAP-1

- BRCA1 associated protein-1 (ubiquitin carboxy-terminal hydrolase)
- Deubiquitinating enzyme encoded by the BAP1 gene
- Encodes an 80.4 kDa nuclear-localizing protein with a ubiquitin carboxy-terminal hydrolase domain that yields activity
- A conserved transcriptional repressor required for long-term silencing of genes that regulate cell fate, pluripotency, etc

BAPomas

- Sporadic or familial (syndromic)
- Common in combined melanocytic nevi
- Not clinically atypical (small, domed, papular, often non-pigmented)
BRAF fusions are initiating events in melanocytic neoplasia

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<thead>
<tr>
<th>Fusion</th>
<th>CR1</th>
<th>CR2</th>
<th>Kinase Domain</th>
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<td>WT BRAF</td>
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</table>

23 year old woman with melanoma in small bowel
CGH summary

- Tumors 0.5 mm thick and above
- Timely; 2 weeks if block readily available
- germane to all the usual suspects
  - Spitz vs. MM
  - DPN vs. MM
  - Dyplasia vs. nevoid MM, etc.

CGH vs. FISH

Skin 2008

Small bowel 2011
Thank you

iwei.yeh@ucsf.edu