Molecular Aspects of Melanocytic Neoplasia

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Outline

• Melanoma oncogenes and implications for treatment
• Assessment of copy number aberrations for diagnostic purposes

Melanocytic Nevi Arise From Initiating Oncogenic Mutations
Initiating Oncogenes in Common Nevi

- BRAF
- NRAS
- Unknown

Pollock et al. Nature Genetics 2003

Initiating Oncogenes in Blue Nevi and Uveal Melanoma

- GNAQ
- GNA11
- Unknown

VanRaamsdonk et al. 2010 NEJM

Initiating Oncogenes in Spitz Tumors circa 2010

- HRAS
- Unknown

Bastian et al. 2000 Am J Pathology

HRAS Spitz Nevus
Spitz Tumor with BAP1 loss

Initiating Oncogenes in Spitz Tumors circa 2012

BRAF + BAP1 loss
HRAS
Unknown

Initiating Oncogenes in Spitz Tumors

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

Wiesner et al. 2014 Nature Communications

ALK Fusion AST
Targeted Therapies

<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>
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</thead>
<tbody>
<tr>
<td>BRAF mutation</td>
<td>vemurafenib, dabrafenib, trametinib</td>
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<tr>
<td>NRAS mutation</td>
<td>MEK162</td>
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<td>KIT mutation</td>
<td>imatinib</td>
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<td>ALK fusion</td>
<td>crizotinib, ceritinib</td>
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<td>RET fusion</td>
<td>cabozaftinib, vandetanib</td>
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<tr>
<td>ROS1 fusion</td>
<td>crizotinib</td>
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<td>BRAF fusion</td>
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Detecting copy number abnormalities:

- FISH (fluorescence in situ hybridization)
- CGH (comparative genomic hybridization)
- NGS (next generation sequencing)

Melanomas Frequently Demonstrate Copy Number Aberrations


Copy number changes for 32 melanomas 94% demonstrated copy number aberrations

FISH: What gets analyzed?
**FISH: Potential effectiveness in melanoma diagnosis**

- 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

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**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

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**What gets FISHed?**

- Spitz vs. spitzoid MM
What gets FISHed?

- Spitz vs. spitzoid MM
- Combined nevus vs. MM ex nevus

What gets FISHed?

- Spitz vs. spitzoid MM
- Combined nevus vs. MM ex nevus
- Acral nevus vs. acral MM

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- Dysplastic nevus vs. nevoid MM

What gets FISHed?

- 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
What gets FISHed?

- 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
- Nevus NOS vs. nevoid MM
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 cells
- 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
CGH (Comparative Genomic Hybridization)

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
Comparative Genomic Hybridization

- Microdissect tumor sections
- Extract DNA
- Tumor DNA
- Normal DNA
- Label DNA
- Hybridize to Microarray
- Image
- Analyze

Comparative Genomic Hybridization (CGH)

- CGH gain
What gets analyzed by CGH?

- Spitz vs. spitzoid MM
- Combined nevus vs. MM ex nevus
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36 year old woman
Spitz vs. 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
60 year old male; back

Indication: diagnostic uncertainty (probably triggered by spitzoid melanocytes in an older patient)
BAP-1

- BRCA1 associated protein-1
- Deubiquitinating enzyme (ubiquitin carboxy-terminal hydrolase)
- Localizes to transcription start sites and modulates transcriptional regulation
Spitz Nevi with BAP1 loss

- Sporadic or familial (syndromic)
- Germline BAP1 loss families with increased incidence of uveal/cutaneous melanoma, renal cell carcinoma, mesothelioma.
- Not clinically atypical (small, domed, papular, often non-pigmented)
- BAP1 loss often observed in the Spitzoid portion of combined nevi

39 yo woman on the back
Indication: diagnostic ambiguity
Botton, Yeh et al. PCMR 2013

MAD1L1-BRAF fusion

Botton, Yeh et al. PCMR 2013
23 year old woman with melanoma of small bowel
No history of melanoma
Thank you
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Initiating Oncogenes in Blue Nevi and Uveal Melanoma

Blue Nevi
Unknown
GNA11
GNAQ

Uveal Melanoma
Unknown
GNA11
GNAQ

VanRaamsdonk et al. 2010 NEJM