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

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

VanRaamsdonk et al. 2010 NEJM
Initiating Oncogenes in Spitz Tumors circa 2010

Bastian et al. 2000 Am J Pathology
HRAS Spitz Nevus
Initiating Oncogenes in Spitz Tumors circa 2012

- BRAF + BAP1 loss
- HRAS
- Unknown

Initiating Oncogenes in Spitz Tumors

- BRAF/NRAS +BAP1
- HRAS
- BRAF Fusions
- NTRK1 Fusions
- ROS1 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>
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<td>MEK162</td>
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<td>KIT mutation</td>
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<td>imatinib</td>
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<td>ALK fusion</td>
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<td></td>
<td>crizotinib, ceritinib</td>
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<td>RET fusion</td>
<td></td>
<td></td>
<td>cabozantinib, vandetanib</td>
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<tr>
<td>ROS1 fusion</td>
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<td>crizotinib</td>
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<td>BRAF fusion</td>
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<td>sorafenib, trametinib</td>
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<tr>
<td>NTRK1 fusion</td>
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<td>cabozantinib</td>
</tr>
</tbody>
</table>
Outline

• Melanoma oncogenes and implications for treatment
• Assessment of copy number aberrations for diagnostic purposes
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
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
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
What gets FISHed?

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

Snijders et al., Nat. Genet. 1998
Our array CGH platform
Agilent 4x180k human array
Comparative Genomic Hybridization

Microdissect tumor sections

Extract DNA

Tumor DNA
Normal DNA

Label DNA

Hybridize to Microarray

Image

Analyze
CGH

Log2 Ratio

1  2  3  4  5  6  7  8  9  10  11  12  13  14  15  16  17  18  19  20  22  XY
CGH

Log2 Ratio

1  2  3  4  5  6  7  8  9  10  11  12  13 14  15  16  17  18  19  20  22  XY

1  2  3  4  5  6  7  8  9  10  11  12  13 14  15  16  17  18  19  20  21  22  X  Y
CGH gain
CGH 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
- Nevus NOS vs. nevoid MM
36 year old woman
Spitz vs. melanoma
Advantages of aCGH

• Entire genome is examined
• Timely (2 week turnaround)
• Expense similar to FISH
CGH vs. FISH

RREB1  MYB  CCND1  CEP6
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
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

VanRaamsdonk et al. 2010 NEJM