Gene expression profiling for melanoma diagnosis and prognosis

Rajendra Singh, MD
Associate Professor
Icahn School of Medicine at Mt. Sinai
New York, NY 10029
skmpathology@gmail.com

• Molecular tests for melanocytic lesions

What are molecular tests available for melanocytic lesions and why do we need them?
TREATMENT DECISIONS

Guide therapeutic decision making
DNA sequencing/Mutation analysis
• BRAF
• NRAS
• GNAQ/GNA11
• cKIT

• Not helpful as diagnostic test
• $500-1000 per gene

• Treatment decisions
• BRAF mutation testing, mutation panels
• Diagnosis
• FISH, CGH, Gene expression profiling, Prognosis
• Gene expression profiling
• V600E/K mutations confer increased sensitivity to BRAF inhibitors (vemurafenib, dabrafenib)

How is BRAF testing performed?
• PCR-based BRAF V600 mutation test
• Used on formalin-fixed, paraffin-embedded tissue, so biopsy specimens sent for routine histopathology are used
• Sensitive detection of the BRAF V600E mutation
• May also detect other mutations such as V600D, V600K, V600R

Additional options for BRAF testing
• Targeted next generation sequencing panels-tests available in different formats testing 50-400 genes
• Ability to assay for mutations in multiple oncogenes
• Used on formalin-fixed, paraffin-embedded tissue
Actionable mutations in metastatic melanoma

- BRAF V600E/K **BRAF and/or MEK inhibitor**
- NRAS **MEK inhibitor**
- KIT **imatinib, dasatinib**

• Treatment decisions
  - BRAF mutation testing, mutation panels
• Diagnosis
  - Gene expression profiling, CGH, FISH
• Prognosis
  - Gene expression profiling

Melanocytic lesions with ambiguous histopathology pose a major diagnostic challenge
What Molecular Tests are Available?

- Fluorescent in situ hybridization (FISH)
- Comparative genomic hybridization (CGH)
- Gene Expression Profile Testing-myPATH

Fluorescent in situ hybridization (FISH)

- Sensitivity 80s-90s%; Specificity 90s%
- 4-8 probes typically used
- Most melanomas demonstrate copy number increases of 11q and 6p, and can be easily distinguished from common nevi

CGH

- Label normal and test DNA with separate dyes
- Competitively hybridize to
  - Metaphase Spread or
  - cDNA array.
- Array-based comparative genomic hybridization is more sensitive than traditional comparative genomic hybridization
- 96% melanomas have genomic aberrations
- 13% nevi have genomic aberrations (Spitz 11p)

**FISH**
- only detect genes and chromosomes targeted by specific probes
- may detect balanced chromosomal translocations and single-point mutations
- Polyploidy can lead to false-positive fluorescence in situ hybridization results
- Priced per probe; total cost ranges $500-2000

**CGH**
- Detects genome-wide DNA differences between test and reference samples
- may reveal chromosomal alterations in cancerous lesions
- False-negative results occur if tumor cells are not adequately represented in sample
- Unable to detect point mutations and balanced translocations
- $1800-6000

---

**Gene Expression Profile Testing**

- myPath Melanoma (Myriad Genetics)
- 23 genes
- 90% sensitivity; 91% specificity
- Primary tumor only
- Price $900

---

**Evaluating benign nevi and melanomas using a gene expression signature**

Candidate biomarker genes identified, based on differential expression in benign vs primary malignant melanocytic lesions reported in the literature or observed in practice
Initially identified 79 candidate biomarker genes narrowed to a smaller set of the 40 most promising biomarkers

*Clarke LE. J Cutan Pathol (2015); 42: 244-252*
Evaluating benign nevi and melanomas using a gene expression signature

Using a training set of 464 melanocytic lesions, a 23 gene signature yields an area under the curve of (AUC) 96%

- RT-PCR of RNA from FFPE tissue
“...a clinically validated test to be used as an adjunct to histopathology when the distinction between a benign nevus and a malignant melanoma cannot be made confidently by histopathology alone.”
(Not FDA approved)

Clinical validation study: Melanoma Diagnostic score

Myriad myPath® melanoma

Melanoma Diagnostic Score

Benign  -16 to -2
Indeterminate  -2 to 0
Malignant  >0-10

Image reproduced from: Clarke LE. J. Cutan Pathol (2015); 42: 244-252.
## Independent Validation Studies

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Agreement with Histopathology</th>
<th>Inter-test agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unequivocal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnostic lesions</td>
<td>FISH</td>
<td>93%</td>
<td>100%</td>
<td>97%</td>
</tr>
<tr>
<td></td>
<td>MyPath</td>
<td>62%</td>
<td>95%</td>
<td>83%</td>
</tr>
<tr>
<td><strong>Challenging</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnostic lesions</td>
<td>FISH</td>
<td>56%</td>
<td>83%</td>
<td>70%</td>
</tr>
<tr>
<td></td>
<td>MyPath</td>
<td>52%</td>
<td>80%</td>
<td>64%</td>
</tr>
</tbody>
</table>

Adapted from Minaca EC et al. Modern Pathology (2016) 29, 832-843

## Sensitivity Analysis

<table>
<thead>
<tr>
<th>Melanoma Subtype</th>
<th>Validation I Sensitivity (n = 201)</th>
<th>Validation II Sensitivity (n = 177)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lentigo maligna melanoma</td>
<td>93.3%</td>
<td>89.7%</td>
</tr>
<tr>
<td>Nodular melanoma</td>
<td>100%</td>
<td>91.4%</td>
</tr>
<tr>
<td>Superficial spreading melanoma</td>
<td>90.9%</td>
<td>94.8%</td>
</tr>
<tr>
<td>All melanomas</td>
<td>94.0%</td>
<td>91.5%</td>
</tr>
</tbody>
</table>

Adapted from Clarke LE et al. Cancer (2016) 00, 1-12
MyPath for Benign vs. Malignant

MyPath
Sensitivity: 62-94.5%
Specificity: 91-95%

Challenging lesions

MyPath
Sensitivity: 52%
Specificity: 80%

FISH
Sensitivity: 56%
Specificity: 83%

- Treatment decisions
  - BRAF mutation testing, mutation panels
- Diagnosis
  - Gene expression profiling, CGH, FISH
- Prognosis
  - Gene expression profiling

Currently, the only prognostic techniques for melanoma are AJCC criteria and Sentinel Lymph Node status

Is it possible to identify patients who will develop more aggressive disease?
GENE EXPRESSION PROFILING

- Genetic Testing to Assist in Prognosis of Melanoma
- FDA approved test developed by Castle Bioscience, Friendswood, Texas
- Uses formalin-fixed, paraffin-embedded tissue
- Quantifies expression of 31 genes from primary tumor to develop a Gene Expression Profile (GEP)
- Applies a validation algorithm to classify patients as Class 1 (low) vs Class 2 (high) risk of developing metastatic disease within 5 years

DecisionsDx® Melanoma: RT-PCR

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Gene Name</th>
<th>Class 1 Ranges</th>
<th>Class 2 Ranges</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCC</td>
<td>E-cadherin</td>
<td>1.011-1.030</td>
<td>1.062-1.084</td>
</tr>
<tr>
<td>ERBB3</td>
<td>ERBB3</td>
<td>1.011-1.030</td>
<td>1.062-1.084</td>
</tr>
<tr>
<td>FHL1</td>
<td>FHL1</td>
<td>1.011-1.030</td>
<td>1.062-1.084</td>
</tr>
<tr>
<td>GPR134</td>
<td>GPR134</td>
<td>1.011-1.030</td>
<td>1.062-1.084</td>
</tr>
<tr>
<td>GSTP1</td>
<td>GSTP1</td>
<td>1.011-1.030</td>
<td>1.062-1.084</td>
</tr>
<tr>
<td>HSPB1</td>
<td>HSPB1</td>
<td>1.011-1.030</td>
<td>1.062-1.084</td>
</tr>
<tr>
<td>KRT14</td>
<td>KRT14</td>
<td>1.011-1.030</td>
<td>1.062-1.084</td>
</tr>
<tr>
<td>LEST1</td>
<td>LEST1</td>
<td>1.011-1.030</td>
<td>1.062-1.084</td>
</tr>
<tr>
<td>MAGEA4</td>
<td>MAGEA4</td>
<td>1.011-1.030</td>
<td>1.062-1.084</td>
</tr>
<tr>
<td>MET</td>
<td>MET</td>
<td>1.011-1.030</td>
<td>1.062-1.084</td>
</tr>
<tr>
<td>MX1</td>
<td>MX1</td>
<td>1.011-1.030</td>
<td>1.062-1.084</td>
</tr>
<tr>
<td>NOS3</td>
<td>NOS3</td>
<td>1.011-1.030</td>
<td>1.062-1.084</td>
</tr>
<tr>
<td>PDE4D</td>
<td>PDE4D</td>
<td>1.011-1.030</td>
<td>1.062-1.084</td>
</tr>
<tr>
<td>PDGFRA</td>
<td>PDGFRA</td>
<td>1.011-1.030</td>
<td>1.062-1.084</td>
</tr>
<tr>
<td>SMAD4</td>
<td>SMAD4</td>
<td>1.011-1.030</td>
<td>1.062-1.084</td>
</tr>
<tr>
<td>SOCS3</td>
<td>SOCS3</td>
<td>1.011-1.030</td>
<td>1.062-1.084</td>
</tr>
<tr>
<td>STK11</td>
<td>STK11</td>
<td>1.011-1.030</td>
<td>1.062-1.084</td>
</tr>
<tr>
<td>TGFBR1</td>
<td>TGFBR1</td>
<td>1.011-1.030</td>
<td>1.062-1.084</td>
</tr>
<tr>
<td>TGFBR2</td>
<td>TGFBR2</td>
<td>1.011-1.030</td>
<td>1.062-1.084</td>
</tr>
<tr>
<td>TIMP3</td>
<td>TIMP3</td>
<td>1.011-1.030</td>
<td>1.062-1.084</td>
</tr>
<tr>
<td>VEGFA</td>
<td>VEGFA</td>
<td>1.011-1.030</td>
<td>1.062-1.084</td>
</tr>
</tbody>
</table>

Class 1 test result:
- Low risk of metastasis within 5 years

Class 2 test result:
- High risk of metastasis within 5 years

Adapted from Gerami P et al, Clin Cancer Res, 2015; 21(1).
Gene Expression Profile Testing

- Prognosis was Independent of Breslow thickness, ulceration status, SLNB, and mitotic index in predicting metastatic disease
- Bill $7900 (avg. collection $1500)

"DecisionDx®-Melanoma molecular test for cutaneous melanoma is a proprietary gene expression assay


Accuracy of class prediction for stage I and II cutaneous melanoma subgroups

Adapted from Gerami P et al, Clin Cancer Res, 2015; 21(1).
Monti Sinai Experience
Case #1
41 year old female with a left forearm lesion
Clinical: BCC vs. poroma
FISH:
  Increased 6q25 RREB (intermediate risk)

Myriad MyPath Melanoma Score:
  3.5 (Benign)
Mount Sinai Experience
Case #2

26 year old with left wrist lesion
Mount Sinai Experience  
Case #2

FISH:  
Loss of 6q23 (MYB), low risk lesion,  
50% SLN+

Myriad MyPath Melanoma Score:  
-10.5 (Benign)

DecisionDx®-Melanoma  
Summary

- independent prognostic value predicting risk of metastasis in Stage I and Stage II
- Sensitivity 85-89% (ROC AUC = 91%)
- Prognostic values (prospective studies) in Class 2
  - 5y Melanoma Specific Survival = 34%
  - 5y DMFS = 46-49%
  - 5y OS = 55-57%

Limitations and  
Future Directions

Critiques/limitations  
- Analytical: RNA quality?
- Primary biopsy vs. Wide local excision
- Multivariate analysis including molecular + histology + regional LN status + clinical data

Future Studies  
- Prospective, blinded studies on change in DFS, DMFS, and OS
- Increased detection rate?
- Improved melanoma specific outcomes?
- Stage III patients
Acknowledgements

• Dr. David Terrano

• THANK YOU

QUESTIONS?