Differentiation of Melanoma from Dysplastic Nevi in Suspicious Pigmented Skin Lesions by Non-invasive Tape Stripping

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DermTech International, Inc.

Society for Investigative Dermatology
2007 Annual Meeting
Plenary Session II
## Conflict of Interest Statement

<table>
<thead>
<tr>
<th>Name</th>
<th>Conflict of Interest</th>
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</thead>
<tbody>
<tr>
<td>William Wachsman</td>
<td>Uncompensated member DermTech Scientific Advisory Board</td>
</tr>
<tr>
<td>Matthew Zapala</td>
<td>N/A</td>
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<td>Don Udall</td>
<td>N/A</td>
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<tr>
<td>Aimee Paik</td>
<td>N/A</td>
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<tr>
<td>Tissa Hata</td>
<td>Research funding from DermTech International</td>
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<td>Lory Walls</td>
<td>N/A</td>
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<tr>
<td>Rita Wong</td>
<td>Employee of DermTech International</td>
</tr>
<tr>
<td>Nicholas Schork</td>
<td>Paid consultant to DermTech International</td>
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<tr>
<td>Sherman Chang</td>
<td>Employee of DermTech International</td>
</tr>
<tr>
<td>Boris Bastian</td>
<td>Paid consultant to DermTech International</td>
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Background and Objectives

• Melanoma
  – 59,940 (estimated) new cases invasive melanoma in US in 2007
  – 1 in 68 lifetime risk
  – Nearly equal number of melanoma in situ cases
  – Incidence rapidly rising
  – Early diagnosis paramount to survival
  – Diagnostic gold standard: histopathology of excision biopsy
  – Only 3-10% of suspicious pigmented lesions are melanoma

• Genomic analyses of melanoma
  – Use of excision biopsies
  – Distinguish melanoma from benign/dysplastic nevi and normal skin

• Study objectives
  – Determine whether stratum corneum RNA harvested by non-invasive tape stripping can be used to distinguish melanoma from dysplastic nevi in suspicious pigmented lesions
  – Develop a multigene classifier for clinical prediction of melanoma
Epidermal Genetic Information Retrieval (EGIR)

- Developed by Morhenn and Rheins at DermTech International
- EGIR RNA used in RT-PCR & microarray assays
- Use of EGIR to analyze psoriasis
- Simple, non-invasive method to harvest stratum corneum RNA
  - 4 x 20 mm tapes harvest 4-7 ng total RNA from normal skin
  - Lesions of 3 mm can be assayed
  - RNA stable at ambient temperature for 72 hr
Clinical Protocol

• IRB-approved protocol at all sites

• Inclusion criteria
  – Subjects 18 or older
  – Pigmented lesion, suspicious for melanoma that requires biopsy
  – Lesion size: 6 mm or greater
  – If 2 lesions, must have > 4 mm separation

• Exclusion criteria
  – Lesion that is ulcerated, bleeding or weeping
  – Use of topical medications or systemic steroids within 30 d
  – Use of topical moisturizer or sunscreen on sites within 24 h
  – Allergy to tape or latex

• Procedure
  – Informed consent obtained
  – Tape stripping of lesion(s) and uninvolved, control skin
  – Demarcate lesion edge on tape
  – Biopsy of lesions as per standard of care
Clinical Protocol

• Dermatopathology
  – Primary review at clinical site
  – Central review performed by Boris Bastian (UCSF)

• Histopathology of specimens
  – Melanoma (15)
    • In situ (10)
    • Invasive (5)
  – Nevi (38)
    • Clark’s (30)
    • Lentiginous (4)
    • Congenital pattern (4)
Experimental Assay Methods

• RNA isolation
  • Use only demarcated part of tape
  • MELT (Ambion)

• RNA QC metrics
  • Experion system: visible 18S band
  • yield ~ 1 ng

• Amplification
  • WT-Ovation Pico RNA System (NuGen)
  • 200-500 pg/reaction

• Amplified cDNA QC
  • yield > 5 mcg
  • size distribution of cDNAs: ~ 1000 nt

• Hu U133 plus 2.0 GeneChip (Affymetrix)
  • ~ 47K unique transcripts

• Array data QC
  • Assess with Simpleaffy from R
  • Scaling factor < 5 (average 2.6)
  • % present calls > 30%
  • average background ~ 30

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Profiling Distinguishes Melanomas from Dysplastic Nevi and Normal Skin

Data Handling
• GCRMA normalization
• Background: 50
• ANOVA (p < 0.05)
• FDR (q < 0.05)
• Multiple testing correction
  • Westfall & Young permutation
• 117 differentially expressed genes

Hierarchical clustering analysis
• log2-transformed, mean-centered for arrays and genes
• cluster with complete linkage metric

D: Dysplastic nevus (n= 38)
M: Melanoma (n= 15)
N: normal skin (n= 12)

Results:
• Melanomas distinguished from normal skin
• At least 3 major groups of dysplasia
• Suggests existence of low and high grade dysplastic nevi

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Functional Characteristics of DE Genes

• Goal
  – Compare genes DE between melanoma and dysplastic nevi

• Strategy
  – Start with 117 DE genes from GEP
  – Filtering criteria
    • t-test (p < 0.01)
    • FDR (q < 0.05)
    • Fold change: 2x difference

• Results
  – 89 DE genes between melanoma and dysplastic nevi
  – Major functional categories
    • Hair/skin development/function (15)
    • Cellular development (18)
    • Cell growth/proliferation (16)
    • Cancer (24)

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<th>No.</th>
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<td>MYO5A</td>
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All 9 genes known to be expressed in melanocytes

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Genes Involved in Skin Development & Function

Melanin Biosynthesis

Melanocyte Differentiation

Melanocyte Proliferation

Melanocyte Development

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Genes Involved in Cell Death & Survival

Apoptosis of melanoma cell lines

Survival of melanoma cells

Transformation of skin cell lines

Malignant tumor
A 5 Gene Classifier Distinguishes Melanoma from Dysplastic Nevi

- Starting set of genes
  - 33 most statistically significant of 89 differentially expressed between melanoma and dysplasia
- Method
  - Stepwise binary logistic regression

### Variables in the equation

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<th>Variable</th>
<th>$\beta$</th>
<th>S.E.</th>
<th>Wald</th>
<th>df</th>
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$\beta$ = the coefficient of the predictor variables
A Wald test is used to test the statistical significance of each coefficient ($\beta$) in the model.

### Training Set

<table>
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<tr>
<th>Observed</th>
<th>Predicted Class</th>
<th>Diagnosis</th>
<th>Melanoma</th>
<th>Dysplastic nevi</th>
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Conclusions

1. EGIR-based, non-invasive tape stripping of stratum corneum can be used to differentiate melanoma from dysplastic nevi

2. Suggests that different classes of dysplastic nevi can be distinguished by gene expression profiling

3. Some of the functions of genes differentially expressed between melanoma and dysplastic nevi are related to melanoma biology

4. Suggests that gene expression profile of stratum corneum is altered, either directly or indirectly, by the presence of melanoma

5. Classifier for class prediction of melanoma vs dysplastic nevi has been developed

6. Preliminary results show that a 5 gene classifier accurately discriminates melanoma from dysplastic nevi

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Acknowledgements

UCSF:
• Boris Bastian - central review of specimen histopathology

Clinical sites:

<table>
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<tr>
<th>No.</th>
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<tr>
<td>1</td>
<td>UCSD, La Jolla, CA</td>
<td>Tissa Hata</td>
</tr>
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<td>2</td>
<td>Skin &amp; Cancer Associates, Plantation, FL</td>
<td>Harold Rabinovitz</td>
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<td>3</td>
<td>Skin Surgery Medical Group, San Diego, CA</td>
<td>Ken Gross</td>
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<td>Dermatology and Advanced Aesthetics, Lake Charles, LA</td>
<td>Shondra Smith</td>
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<td>Dermatology Research Associates, Los Angeles, CA</td>
<td>Howard Sofen</td>
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<td>Dermatology Associates, Florence, KY</td>
<td>James Zalla</td>
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<td>Solano Dermatology Associates, Vallejo, CA</td>
<td>Serena Mraz</td>
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<td>Bernard Goffe</td>
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<td>9</td>
<td>UC Davis, Sacramento, CA</td>
<td>Fu-tong Liu</td>
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<td>Raoof Laser &amp; Dermatology Center, Encino, CA</td>
<td>Joseph Raoof</td>
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<td>11</td>
<td>Oregon Dermatology &amp; Research Center, Portland, OR</td>
<td>Phoebe Rich</td>
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<td>Saltzer Medical Group, Nampa, ID</td>
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<td>David Pariser</td>
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<td>Roy Geronemus</td>
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<td>Daniel Siegel</td>
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DermTech International:
• Cheryl Peters
• Louise Lisansky
• George Schwartz

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