Introduction

Melanoma incidence is increasing in the US population faster than all other cancers. It is already the seventh most common cancer in the United States with a lifetime risk of 1 in 41 in men and 1 in 61 in women. Melanoma accounts for more than 70% of skin cancer deaths, but when detected early it is considered highly curable. In current clinical practice, the detection of melanoma is based upon visual cues, including the "ABCDE" criteria for pigmented nevi and results of optical imaging techniques, such as dermoscopy and confocal microscopy. However, only 2 to 10% of lesions biopsied for suspicion of melanoma are positive for this diagnosis. In addition, differences in histopathologic review of biopsy specimens by experienced dermatopathologists can result in discordant tissue diagnoses for 10-35% of melanomas. Thus, there is a need for a test that amplifies the accuracy, objectivity and ease of detection in clinical practice.

The patented Epidermal Genetic Information Retrieval (EGIR) technology (DermTech International, Inc.) uses a controlled adhesive film to non-invasively obtain RNA from stratum corneum (i.e. the uppermost layer of the skin). Developed by Morhenn and Rhinos (J Am Acad Dermatol 4: 687. [1999]), the EGIR methodology uses four 20 mm tapes applied to the skin to non-invasively sample stratum corneum. RNA so harvested has been found to be stable for assay, when kept at ambient temperature for at least 72 hours. This makes it an ideal method for use in the clinical setting.

Previous work showed that EGIR, non-invasive tape stripping of stratum corneum, identified 284 genes that differentiated melanoma from atypical nevi and normal skin (p<0.001, false discovery rate q<0.05). (Wachsman et al. [2008])

Methods

Materials, Methods and Clinical Protocol

**Materials**

- 1,000 melanoma cases collected at 9 centers in U.S.
- 180,000 μl RNA isolated at -80°C
- 3077 (Ambion, Inc.) used to extract RNA
- Shipped and stored at -80°C
- Assay methods
  - Training set: TreeNet used for class prediction
  - GCRMA used to normalize data
  - Data QC using Yield and quality assayed
  - Only used tape demarcated over pigmented lesion
  - If 2 lesions, must have > 4 mm separation

**Methods**

- 24 lesions randomized to training and test sets
- 89 nevi randomized to test sets
- 37 melanomas and 37 nevi
- 37 melanomas from 8 centers
- 89 nevi from 4 centers
- Basal cell carcinoma (18)
- Seborrheic keratosis (16)
- Actinic keratosis (9)
- Lentigo maligna (3) & lentigo maligna melanoma (1)
- Superficial spreading melanoma (32): in situ (11) and invasive (21)
- Lentigo maligna/lentigo maligna melanoma (LM/LMM) and solar lentigo
  - Solar lentigines (12)
  - Lentigo maligna (N=5), lentigo maligna melanoma (N=5) and solar lentigo (N=12)
- Nevus (89): Blue nevus (1), Clark (67), congenital (12) and lentiginous (9)
- Nevus (37): Clark (29) and lentiginous (8)
- Primary and central
  - Demarcate lesion edge on tape
  - Tape stripping of lesion(s) and uninvolved, control skin
- Subjects 18 or older
- If multiple lesions, > 4 mm spacing

**Clinical Protocol**

- Collection: Subjects 18 or older
- Multiple lesions, > 4 mm spacing
- Specimens: pre-amplified with WT-Ovation FFPE kit (NuGen, Inc)
- Translation of the Assay from Microarray to qRT-PCR Platform
- Goals: To develop a classifier to differentiate U/L/W/MMI from solar lentigo by EGIR-based genomic analysis
- Methods
  - EGIR tape-based specimens were expression profiled by GeneChip assay
  - GeneChip data were analyzed by TreeNet for class selection and prediction
  - TreeNet analysis for class selection and prediction
  - TreeNet and GCRMA for class selection
  - Control data obtained using the GeneChip microarray platform

**Characterization of a 19-Gene Classifier that Identifies Melanoma in Pigmented Lesions**

<table>
<thead>
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<th>Test Set</th>
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**Analysis of False Positive Nevi Identified by the 19-Gene Classifier**

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**Conclusions**

- EGIR-E300 assay platform was expression profiled by GeneChip assay
- TreeNet and GCRMA for class selection and prediction
- Control data obtained using the GeneChip microarray platform
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**A 32-Gene Classifier Differentiates Solar Lentigo from Lentigo Maligna/Lentigo Maligna Melanoma**

- Lentigo maligna/malignant nevi, 3,000 MDQ and solar lentigos are often difficult to distinguish on sun damaged skin
- Objective: To develop a classifier to differentiate U/L/W/MMI from solar lentigo by EGIR-based genomic analysis
- Methods
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