DEVELOPMENT OF A GENE EXPRESSION SIGNATURE TO DIFFERENTIATE MALIGNANT MELANOMA FROM BENIGN MELANOCYTIC NEVI

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OBJECTIVE

Develop a gene signature to accurately and objectively differentiate malignant melanoma and benign nevi.

METHODS

Melanocytic Tumor Diagnosis

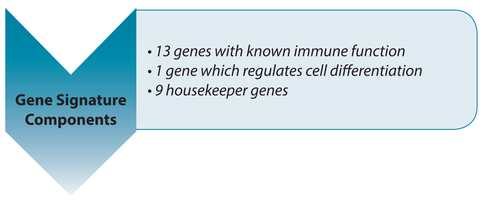
- Initial diagnosis was obtained from the pathology report accompanying each sample.
- Diagnoses were confirmed by an independent, blinded review by a board-certified dermatopathologist.
- Discordant diagnoses were adjudicated by a third independent board-certified dermatopathologist.
- Tumors identified as 'false' negatives by the assay underwent additional diagnostic review by a panel of seven expert dermatopathologists blinded to previous diagnoses and assay score.

Gene Signature Discovery & Development

- Identified 79 potential gene biomarkers with expression profiles shown to vary in melanoma and/or other cancers.
- Used quantitative reverse transcription polymerase chain reaction (qRT-PCR) to measure expression of the 79 genes in 31 melanomas and 52 nevi.
- Narrowed the panel of candidate genes to 40 based upon 1) ability of each gene to differentiate (AUC >70%) benign from malignant lesions and 2) technical reliability.
- Assessed expression of the 40 candidate genes in 544 melanocytic lesions by qRT-PCR (272 melanomas & 272 nevi).
- Used forward selection in a logistic regression model to identify the subset of genes which most effectively discriminate benign from malignant lesions.
- A refined logistic regression model was then used to generate a single score capable of differentiating benign nevi from malignant melanoma. (Figure 1A).

Figure 1A. Model Development

Figure 1B. Genes included in Gene Signature



RESULTS

Cohort Design

- The final data set consisted of a sample cohort of 464 melanocytic lesions (254 melanomas & 210 nevi) representative of all major histopathologic subtypes (Table 1).
- 80 samples failed to generate a score and were excluded from final analysis.
  - RNA degradation in archived samples (>5 years old) was a common cause of failure.

Table 1. Melanocytic Lesions by Subtype

Type	'Ordinary' nevus	Dysplastic / nevus	Blue nevus	Spitz nevus	Solar lentigo	Overall
N	**47**	**117**	**64**	**38**	**6**	**272**
Male	29	48	24	24	1	126
Female	18	69	40	14	5	146
Median age	50	43	46	21	71	43

Type	SSM	Nodular	Acral	LM / LMM	Other	Overall
N	**179**	**25**	**20**	**42**	**6**	**272**
Male	101	8	11	25	5	150
Female	78	17	9	17	1	122
Median age	58	57	77	68	70	61

SSM: superficial spreading melanoma; LM/LMM: lentigo maligna/lentigo maligna melanoma

RESULTS

Finalized Gene Signature

- Used a 23 gene signature to produce a score for each case (Figure 1B).
- The gene signature score discriminated melanoma from nevi with 89% sensitivity and 93% specificity (p-value=$2X10^{-63}$; AUC = 95%) (Figure 2A).
- Final distribution of scores ranged from -14.9 to +9.6 (binary cutoff at 0) (Figure 2B).
- During a blinded, independent review by seven expert dermatopathologists, 10 of 24 (42%) 'false' negative cases were given a majority diagnosis of benign.

Figure 2. Assay Performance

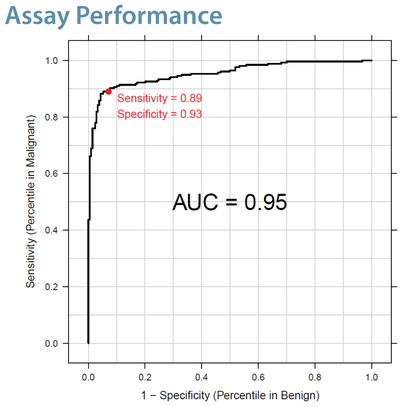


Figure 2A. ROC Curve Demonstrating Sensitivity and Specificity of Assay

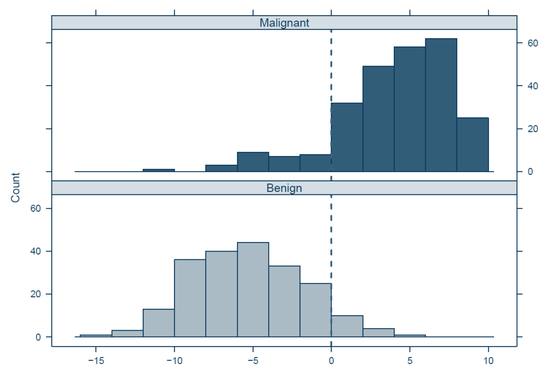


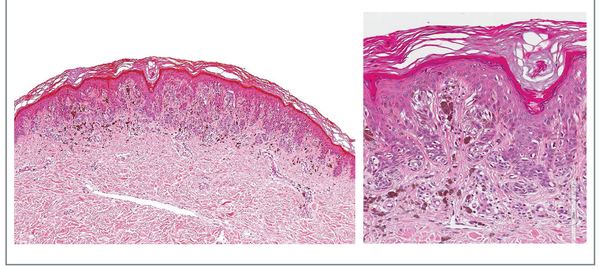
Figure 2B. Distribution of Gene Expression Scores

CASE STUDIES

Case 1. Initial Diagnosis: Superficial spreading melanoma

Panel Diagnosis: Nevus

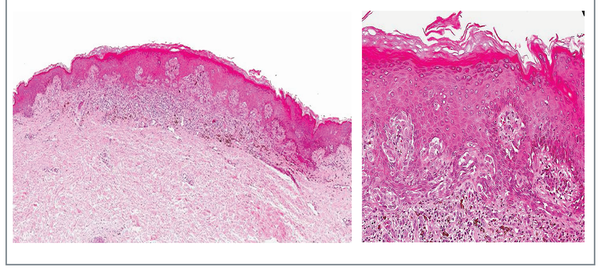
Score: **Benign (- 4.68)**



Case 2. Initial Diagnosis: Superficial spreading melanoma

Panel Diagnosis: 3 Benign / 3 Indeterminate / 1 Malignant

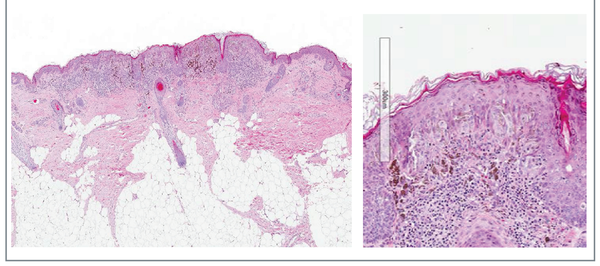
Score: **Benign (- 4.53)**



Case 3. Initial Diagnosis: Spitz nevus

Panel Diagnosis: Spitz nevus

Score: **Malignant (+2.02)**



Case 3 Discussion. While this lesion may be benign, histopathologic examination alone (even by a panel of experts) has been shown to be an unreliable predictor of biologic behavior in tumors such as these (Cerroni et al, Am J Surg Pathol 2010). The malignant score might prompt more thorough review of the case and more conservative management.

CONCLUSIONS

- The identified gene signature, which includes a regulator of cell differentiation and immune response signaling genes, is capable of differentiating benign nevi from malignant melanoma.
- The signature includes genes with known immune function and genes known to regulate cell differentiation.
- Many 'false' negatives identified by the gene signature are thought to be true negatives by an expert panel indicating assay performance may be better than estimated.
- The final gene signature provides additive diagnostic information that allows for a more informed diagnosis of melanocytic lesions.
- Results of this study have recently been confirmed in an independent, clinical validation cohort.

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