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).

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^-6; 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.

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)

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.