CLINICAL VALIDATION OF A GENE EXPRESSION SIGNATURE THAT DIFFERENTIATES BENIGN NEVI FROM MALIGNANT MELANOMA

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BACKGROUND

- Currently, histopathologic evaluation is considered the ‘gold standard’ for the diagnosis of melanocytic lesions; however, many studies demonstrate difficulties in determining a diagnosis when histopathology is used alone.
- The quantitative measurement of biomarker gene expression has been proposed as an adjunctive diagnostic method in the evaluation of ambiguous melanocytic lesions.
- This study aimed to clinically validate a 23-gene expression signature capable of accurately and objectively differentiating malignant melanoma and benign nevi.

METHODS

Sample Cohorts
- All testing was performed on archival FFPE tissue sections of melanocytic lesions.
- Specimens included a broad spectrum of clinical and histopathologic subtypes (Table 1).
- Each case underwent review by an independent dermatopathologist blinded to the initial diagnosis recorded on the pathology report. If there was discordance, the case was adjudicated by a third expert dermatopathologist.

Quantification of Gene Expression
- H&E stained slides were reviewed by an anatomic pathologist and the representative area of each lesion was macro-dissected.
- Total RNA was extracted and gene expression was assessed by qRT-PCR.

Gene Signature Discovery & Verification
- Published literature was reviewed to identify candidate genes with differential expression in melanoma or other cancers.
- The panel of candidate genes was refined based upon 1) ability of each gene to differentiate (AUC > 70%) benign from malignant lesions and 2) technical reliability in a cohort of 83 melanocytic lesions.
- Expression levels were subjected to forward selection in a series of logistic regression models to identify the subset of genes which most effectively discriminate benign from malignant lesions in a training set of 464 samples.
- Genes from similar biological pathways that exhibited correlated expression in the training cohort were consolidated by averaging.
- A refined logistic regression model was used to generate a single numeric score, the Melanoma Diagnostic Score (MDS). A threshold value of zero was applied to the MDS in order to classify malignant melanomas dichotomously as benign or malignant.

Validation of the Melanoma Diagnostic Score (MDS)
- Performance of the test was clinically validated in a second, independent cohort of 437 melanocytic lesions.
- The MDS was validated to differentiate benign nevi from malignant melanoma with a sensitivity of 90% and a specificity of 91%.

RESULTS

- 79 candidate genes were identified based on a review of published literature. These genes were evaluated for differential expression in melanoma and nevus samples using qRT-PCR in a discovery cohort of 83 melanocytic lesions (Figure 1A).
- 40 genes were chosen for further assessment in a larger training cohort of 464 melanocytic lesions.
- Two different groups of strongly correlated genes were observed (cluster 1 and cluster 2), with the remaining genes forming a third loose cluster (cluster 3) (Figure 1B).
- Forward selection in logistic regression models identified the most effective genes for differentiating benign and malignant lesions (Figure 1B).

Table 2. List of Genes in Final Signature

- Incorporating additional gene groups or other individual genes to the model did not increase the diagnostic power.
- The final signature consists of 23 genes (Figure 2, Table 2).
- Performance of the gene signature was clinically validated in a cohort of 437 lesions (Figure 1C).
- The final MDS distribution ranged from -16.7 to +11.1 (Figure 1C).
- Scores from -16.7 to – 0.1 were reported as benign.
- Scores from 0 to +11.1 were reported as malignant.
- The MDS was validated to differentiate benign nevi from malignant melanoma with a sensitivity of 90% and a specificity of 91%.

CONCLUSIONS

- A 23-gene signature has been clinically validated to differentiate melanoma and nevi with a sensitivity of 90% and a specificity of 91%.
- Expression of genes regulating melanocyte differentiation and immune responses appear to represent critical differences between benign and malignant lesions.
- In order to provide a better interpretation of the diagnostic score, an indeterminate zone could be introduced.
- The performance, objectivity, reliability, and minimal tissue requirements of this diagnostic test make it well-suited for clinical use as an adjunct to histopathology in the evaluation of melanocytic lesions.