

## **A Nine-Gene Signature, Expressed in Fresh and FFPE Primary Tumor Tissue, Predicts Clinical Outcome in Malignant Melanoma**

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Conventional histopathological and clinical staging is largely inadequate for predicting clinical outcome of malignant melanoma for all patients. Molecular prognostic markers are not available. We identified a nine-gene signature which is closely associated with the overall survival of melanoma patients.

To identify prognostic genes we correlated the gene expression profiles of 136 primary melanomas with patient overall survival using Cox regression analysis: Based on previous gene expression profiling data, we analyzed the expression of 92 candidate genes in 38 primary melanomas (training cohort), using real-time reverse-transcriptase polymerase chain reaction (RT-PCR). Expression of selected genes was analyzed in an extended group of 91 primary melanomas (study cohort). The resulting prognostic gene signature was validated using an independent set of 45 primary melanomas (validation cohort).

Expression of 11 out of 92 candidate genes correlated with patient survival in the training cohort. A risk score, based on the expression of nine of these genes (KRT9, SPINK7/ECG2, KBTBD10, DCD, HES6, COL6A6, PIP, SCGB1D2, SCGB2A2) or any subgroup thereof, predicted patient overall survival in the study cohort ( $p = 0.0004$ , hazard ratio 3.83), independently of conventional AJCC 2002 staging. Almost all patients (study cohort: 95.4%, validation cohort: 93.8%) with low-risk score and low/intermediate-risk AJCC stage were classified correctly as long-term survivors.

The prognostic value of the signature-based score is its ability, by complementing conventional AJCC staging, to precisely define the need for therapy. Thus, two thirds of patients (29/45, 64%) with intermediate AJCC-based prognosis were reclassified, based on a low-risk score, to a long-term survival probability of

approximately 95%. All other patients were classified, based on a high-risk score, as being at risk of relapse and are, therefore, in need of therapy beyond today's standard care.

Successful translation of our prognostic risk score into clinical practice requires applicability to formalin-fixed, paraffin-embedded (FFPE) primary melanomas. We have developed a technological platform, consisting of (i) total RNA isolation from FFPE tissue samples, (ii) reverse transcription, (iii) cDNA pre-amplification and (iv) real-time RT-PCR using small amplicons, as well as normalization (v) of PCR amplification to a universal reference RNA and (vi) of gene expression to four different house-keeping genes. Using this experimental procedure we demonstrated that gene expression in FFPE melanoma tissue samples (study and validation cohort) correlated with that in corresponding fresh tissue samples, demonstrating applicability of our risk score to FFPE primary melanomas.