Genes and biological functions governing intrinsic tumor biology utilized in cutaneous melanoma risk assessment through a clinically available 31-gene expression profile test

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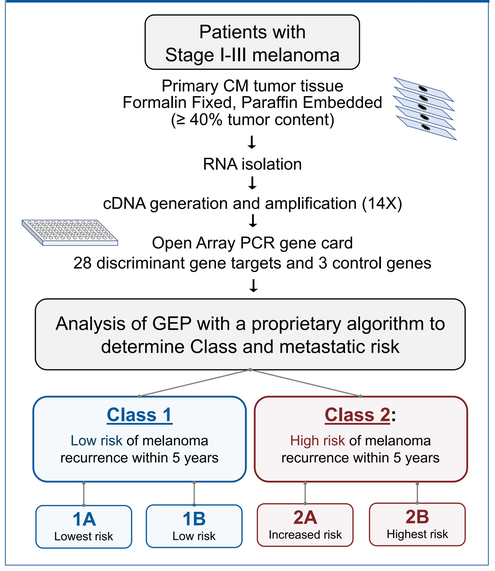
ABSTRACT

Better understanding of intrinsic tumor biology has permitted the development of clinical molecular tests that are objective prognostic tools for various cancers, including cutaneous melanoma (CM). A previously validated 31-gene expression profile (31-GEP) test utilizes RT-PCR of primary CM tumors to predict a patient's risk of recurrence, including sentinel lymph node, locoregional, and distant metastasis events, within 5 years. To develop the test, candidate genes identified in published melanoma gene expression datasets were evaluated for consistency across multiple studies. Herein, we review the methods and data utilized during the development of the 31-GEP test and the known functions of its 28 prognostic genes. Using pathway and protein-protein interaction databases along with literature searches, we demonstrate that the genes assessed by the test are functional components of key melanoma- and cancer-relevant biological processes known to contribute to progression and metastasis and are supported by other studies. The genes utilized to assess melanoma risk play significant roles in processes such as cell-cell communication, differentiation, growth regulation, and immune signaling. These findings suggest that many biological processes, rather than a few pathways, contribute to melanoma progression. Thus, capturing these diverse biological events is necessary for accurate prognostication. In conclusion, the 31-GEP test determines risk by assessing key biological processes associated with progression. Evaluating melanoma tumor biology at a molecular level, in addition to histopathological features, identifies high-risk patients who otherwise would be deemed at low risk for recurrence and metastasis by traditional staging methods alone. Furthermore, these genes could be candidates for novel therapeutic interventions.

BACKGROUND

Workflow for 31-GEP testing

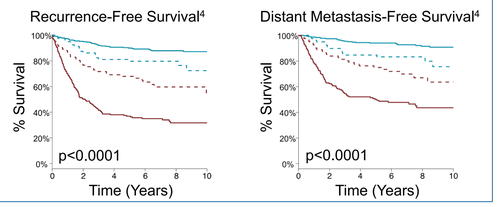


- Exploitation of the intrinsic biology of cancer tissues has permitted the creation of molecular tests that serve as objective diagnostic, prognostic, and therapeutic prediction tools compared to traditional, often subjective methods such as histological and pathological assessments.
- The 31-GEP test predicts a CM patient's risk of recurrence, metastasis, or melanoma- specific mortality at 5 years after diagnosis.
- Patients with a Class 1A and 2B 31-GEP results have the lowest and highest risk, respectively.
- The 31-GEP test is performed in a CAP-accredited/CLIA-certified laboratory using high-throughput RT-PCR assays as previously described[1-4].

Levels of evidence for molecular tests involve grading across 3 categories.
The 31-GEP test has strong evidence in all 3:

CLINICAL VALIDITY

Evidence from retrospective and prospective studies supports consistent ability of the 31-GEP test to accurately identify recurrence, metastasis, and melanoma-specific mortality in CM patients[1-8].



CLINICAL UTILITY

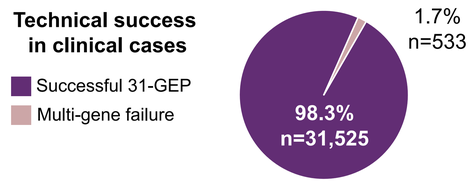
Design (n)	31-GEP Impact
Prospectively tested patients, Retrospective chart review; (156 patients)[9]	53%
Prospective documentation of pre and post test plans; (247 patients)[10]	49%
Prospectively tested patients, Retrospective chart review; (90 patients)[11]	52%
Physician survey of clinical decisions with or without test results; (169 physicians)[12]	47-50%
Physician survey of clinical factors that affect use of 31-GEP test; (181 physicians)[13]	*
*overall GEP impact not assessed with study design	

Data from 3 studies and 2 physician surveys indicate that the 31-GEP test results significantly impact management decisions for approximately 1 of 2 patients[9-13].

ANALYTICAL VALIDITY

The 31-GEP test has high technical reliability on >32,000 clinical cases with adequate tumor content since 2013[14]. Technical success studies demonstrate 99% inter- and 100% intra-assay concordance[15].

Technical success in clinical cases



RESULTS

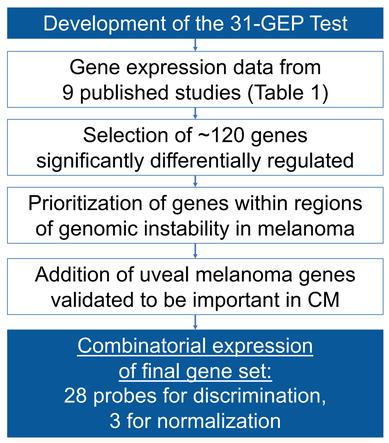


Table 1. Studies utilized for original gene selection during development of the 31-GEP test

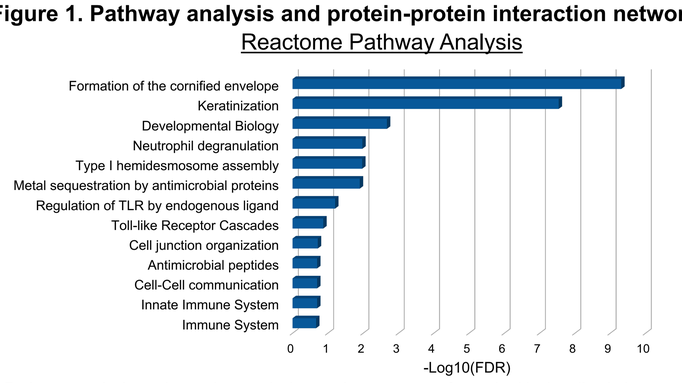
Tissues compared (Tissue Source)	Gene Expression Analysis Platform
Melanocytic nevi, primary melanomas, metastatic melanomas (Microdissected fresh frozen tissue)[16]	Affymetrix Human Genome U133A 2.0 GeneChip
Primary melanomas, melanoma metastases (Laser-capture microdissected cells)[17]	Affymetrix Human Genome U133A array
Melanoma tumor biopsies and cell cultures, normal controls (frozen tumor biopsies)[18]	Microarray
Melanocytic nevi, primary melanomas, melanoma metastases (frozen tumor biopsies)[19]	Research Genetics microarray
Nevi, radial & vertical growth phase melanomas, metastases (fresh tissue)[20]	Agilent Human Whole Genome Oligo Microarray
Normal skin, benign nevi, atypical nevi, early-stage melanoma, advanced-stage melanoma (frozen tissue)[21]	Affymetrix Human Genome U133 Plus 2.0 GeneChip
Vertical growth phase melanomas and distant metastasis (fresh tissue)[22]	Micro-SAGE libraries
Primary melanomas and cutaneous/lymph node metastases (fresh frozen tissue)[23]	Agilent Whole-Human-Genome 44K oligonucleotide array
Primary uveal melanoma with long-term clinical follow-up (fresh tumor samples)[24]	Affymetrix Hu133A and B arrays

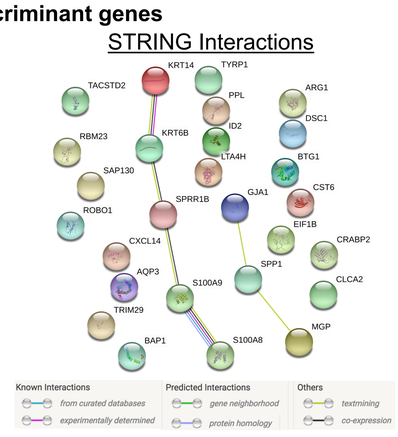
Table 2. Discriminant genes included in the 31-GEP test to assess risk of metastasis

Gene Symbol	Gene Name	Direction of regulation in Class 2	P[a]
AQP3	Aquaporin 3 (Gill blood group)	Down	5.08 e-06
ARG1	Arginase 1	Down	1.05 e-08
BAP1[b]	BRCA1-associated protein-1	Down	0.007
BTG1	B-cell translocation gene 1, antiproliferative	Down	0.024
CLCA2	Chloride channel accessory 2	Down	1.02 e-08
CRABP2	Cellular retinoic acid binding protein 2	Down	0.0006
CST6	Cystatin E/M	Down	1.02 e-08
CXCL14	Chemokine (C-X-C motif) ligand 14	Down	3.31 e-12
DSC1	Desmocollin 1	Down	7.00 e-09
EIF1B	Eukaryotic translation initiation factor 1B	Up	0.024
GJA1	Gap junction protein, alpha 1, 43 kDa	Down	0.034
ID2	Inhibitor of DNA binding 2, dominant negative helix-loop-helix protein	Down	3.91 e-06
KRT14	Keratin 14	Down	1.75 e-05
KRT6B	Keratin 6B	Up	0.16
LTA4H	Leukotriene A4 hydrolase	Down	0.0001
MGP	Matrix Gla protein	Down	0.486
PPL	Periplakin	Down	5.59 e-11
RBM23	RNA-binding motif protein 23	Down	0.018
ROBO1	Roundabout, axon guidance receptor, homolog 1 (Drosophila)	Down	0.0004
S100A8	S100 calcium-binding protein A8	Down	0.031
S100A9	S100 calcium-binding protein A9	Down	0.012
SAP130	Sin3A-associated protein, 130 kDa	Down	0.024
SPP1	Secreted phosphoprotein 1	Up	6.08 e-16
SPRR1B	Small proline-rich protein 1B	Down	0.001
TACSTD2	Tumor-associated calcium signal transducer 2	Down	0.037
TRIM29	Tripartite motif containing 29	Down	2.34 e-09
TYRP1	Tyrosinase-related protein 1	Down	2.41 e-06

[a]p-value reflects t-test analysis of dCt values from non-metastatic cases compared with metastatic cases within the initial training and validation sample cohort. [b]Two assays for BAP1 were included to target both the 5' and 3' regions of the gene.

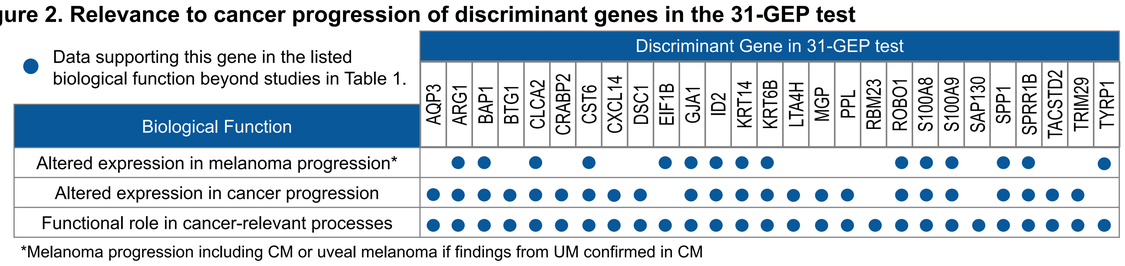
Figure 1. Pathway analysis and protein-protein interaction network of discriminant genes





Pathway analysis and protein-protein interactions were performed with the discriminant genes included in the 31-GEP test using Reactome and STRING, respectively. Pathways with at least 2 entities included and an false discovery rate (FDR) of ≤0.5 are shown.

Figure 2. Relevance to cancer progression of discriminant genes in the 31-GEP test



*Melanoma progression including CM or uveal melanoma if findings from UM confirmed in CM

CONCLUSIONS

- Genes utilized in the 31-GEP test to assess melanoma risk are important in tumor biology, including cell-cell communication and immune signaling. Pathway and predicted interaction analyses suggest that many biological processes, rather than a few pathways, contribute to melanoma progression. Thus, capturing these diverse biological events is necessary for accurate prognostication.
- Many of the genes in the 31-GEP test have been functionally characterized in melanoma, and other genes have documented differential expression contributing to metastasis in other cancers, including some with prognostic significance.

FUNDING & DISCLOSURES

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