

Chapter 2

Melanoma Genomics

Mohammed Kashani-Sabet

Abstract Remarkably, 10 years have elapsed since the initial paper describing genomic profiles of melanoma (Nature 406:536–540, 2000). Since that initial publication, several additional studies have been published examining the potential utility of gene expression profiling of melanoma using a plethora of different technological platforms, data sets, and tissue collections. These studies have provided significant new insights into several aspects of melanoma biology. These include the development of molecular diagnostics assays based on gene expression profiles that are nearing clinical application. As a result, the time is ripe to review the literature regarding gene expression profiling of melanoma. This review will focus on the insights gained in the transcriptomic analysis of (1) distinct phases of melanoma progression; (2) primary melanoma; and (3) metastatic melanoma.

Keywords Gene expression profiling • Microarray analysis • Biomarkers

Gene Expression Profiling of Distinct Phases of Melanoma Progression

Melanoma is an ideal clinical model to which genomic analyses can be applied to study tumor progression because of the readily defined clinical phases of melanoma progression that have histological correlates. The classical model of melanoma progression, defined by Clark et al. [2], described these successive stages, and has represented an important framework for the identification of novel melanoma progression genes. In this model, benign melanocytic proliferation yields a nevus, which can transform into primary melanoma, beginning in the radial growth phase,

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with a capacity to progress to the vertical growth phase, and culminating in metastatic melanoma. While melanoma development is not absolutely dependent on the progression through each of these distinct phases (e.g., melanomas can develop without the presence of preexisting nevi), the vertical growth phase has been postulated to be required for metastatic competency. While this model of melanoma progression was described several years ago and is well understood, prior to the advent of microarray analysis, one would be hard pressed to identify genes whose differential expression corresponded to any of these phases of melanoma progression. In addition, it is important to note that, while BRAF mutations appear to represent an important event in melanoma progression [3] and appear to represent an attractive target for melanoma therapy [4], the high prevalence of BRAF mutations in nevi suggests that they are selected for early in melanoma development. As a result, BRAF mutations are unable to distinguish between the successive phases in melanoma progression as described by this classical model.

To date, several studies have examined the comparative gene expression profiles of melanoma progression. The first published study by Haqq et al. [5] assigned separate gene expression profiles to these distinct phases of melanoma progression using cDNA microarray analysis. Thus, statistical analysis of microarrays (SAM) was able to distinguish between nevi and primary melanomas, and primary and metastatic melanomas. Intriguingly, microarray analysis of radial vs. vertical growth phase melanoma demonstrated exclusive loss of gene expression in the vertical growth phase, and also showed that the gene expression profile characteristic of radial growth phase melanoma was recapitulated in a subset of metastases. These studies suggested that the gene expression signature for metastasis was present in the radial growth phase, challenging the conventional dogma that radial growth phase melanomas are incapable of metastasis.

In addition, unsupervised hierarchical analysis was able to accurately discriminate between a small number of freshly acquired primary melanomas and nevi. This suggested that gene expression profiles could be useful as an adjunct to the pathological diagnosis of melanoma, which has been shown to be discordant in a relatively high percentage of cases [6]. Recently, five markers derived from that microarray analysis were selected for further validation and incorporation into an immunohistochemical multi-marker assay for melanoma [7]. This multi-marker assay was shown to have a high degree of accuracy in the diagnosis of melanocytic neoplasms, with a specificity of 95% and a sensitivity of 91% in a training set of 534 nevi and primary melanomas, comprising the largest analysis of molecular markers in melanoma to date. Subsequently, the multi-marker assay was subjected to four validation sets with greater relevance to the differential diagnosis of nevus vs. melanoma, and found to accurately diagnose a high percentage of melanomas arising in a nevus, Spitz, and dysplastic nevi. Finally, the multi-marker assay was able to correctly diagnose 75% of misdiagnosed neoplasms, suggesting that these markers could help prevent or correct a high percentage of diagnostic errors using routine histopathology of melanocytic neoplasms. This is one of the first demonstrations of the utility of genomic profiles to resolve a differential diagnosis in the realm of oncology, and one of the first successful validations of gene expression profiles

Table 2.1 Selected validated differentially expressed genes between nevi and primary melanoma

Gene	Platform	Gene bank accession number	References
ARPC2	RNA, IHC	NM_005731	[5, 6]
CDH3	RNA	NM_001793	[6, 7]
FN1	RNA, IHC	NM_212482	[5, 6]
KNSL5	RNA	NM_138555	[6, 7]
PLAB	RNA	NM_004864	[6, 7]
PRAME	RNA	NM_206954	[6, 10]
RGS1	RNA, IHC	NM_002922	[5, 6]
SPP1	RNA, IHC	NM_001040058	[5–7]
WNT2	RNA, IHC	NM_003391	[5, 6]

IHC immunohistochemistry

using immunohistochemical techniques. Following further validation in distinct cohorts, it is anticipated that this assay will be available for clinical use.

Subsequently, additional studies investigating the differential gene expression signature of melanoma progression were reported. Three studies have examined the transition between nevus and melanoma. Talantov et al. [8] analyzed 45 primary melanomas, 18 benign nevi, and 7 normal skin specimens analyzed using the Affymetrix platform. Hierarchical clustering was able to identify a unique signature for melanomas. Among the genes overexpressed in melanomas were SPP1 (osteopontin), PLAB, SEMA3B, NES, MAP3K12, and DUSP4. Intriguingly, several genes were identified that were previously shown to be differentially expressed in the Haqq et al. study [5], including SPP1, PLAB, CDH3, KNSL5, CITED1, CSTB, and PSEN2. Separately, Nambiar et al. [9] profiled 11 congenital nevi, 10 primary and 11 metastatic melanomas using the Affymetrix platform. They also identified gene signatures that distinguished nevi from primary melanomas. Upregulated in melanomas were MAPK1, STAT3, ASK/Dbf4, CCNA1, CCNB1, CCNE2, CXCL1, and MCM4. Among the genes downregulated in melanomas were SPON1 and IL-18.

Finally, Koh et al. [10] used a custom array containing 1,100 selected genes to search for differential gene signatures in a tissue set of 120 lesions. They observed higher expression of PHACTR1, HLA-A, HLA-B, PRAME, and STAT1 in melanomas, with higher expression of PTN, GPX3, FABP7, DLC1, and GSTM2 in nevi. The use of these gene signatures was shown to have concordance rates of 90% for melanoma and 86% for nevi. Thus, it is clear that distinct gene expression profiles exist for nevi vs. melanomas, and that these differential gene signatures can be used to assist in this potentially problematic differential diagnosis. A list of novel diagnostic markers for melanoma consistently appearing in several of these studies appears in Table 2.1.

Additional work has focused on exploring the transition between primary and metastatic melanoma. Jaeger et al. [11] profiled 19 primary and 22 metastatic melanomas using Affymetrix oligonucleotide microarrays, and identified 308 genes with differential expression between primary and metastatic lesions. The upregulated genes included SPP1, CDC6, CDK1, mitosin, and fibronectin, whereas E-cadherin, FGFBP, desmocollins 1 and 3, and CCL27 were downregulated. The differential

expression of selected genes in metastatic vs. primary melanomas was validated by immunohistochemical analysis. Using support vector machines, greater than 85% correct classification was achieved between the two types of lesions. Intriguingly, characteristic gene expression profiles were identified in superficial spreading vs. nodular melanomas, and in thin (<1.0 mm) vs. intermediate/thick tumors (>2.0 mm). This is similar to the results identified by Riker et al. [12], who identified a transition point in global transcript profiles, in which melanomas greater than 2 mm in thickness had a gene expression pattern similar to metastases.

Gene Expression Profiles of Primary Melanoma

Due to the paucity of freshly acquired primary melanoma specimens, only a few studies examining the genomic signatures of large numbers of primary melanoma have been performed. In the first study in this category, Winnepenninckx et al. [13] profiled 58 primary melanomas with at least 4 years of follow-up or relapse/death using a pangenomic 44,000 60-mer oligonucleotide microarray. The investigators identified 254 genes that were associated with distant metastasis-free survival, which included genes involved in activating DNA replication origins, e.g., minichromosome maintenance genes and geminin. Twenty-three of these genes were examined at the protein level using immunohistochemical analysis, and found to be associated with overall survival in an independent set. These included MCM3, MCM4, MCM6, KPNA2, and geminin. In a multivariate analysis that included thickness, age, ulceration, and sex, MCM4 and MCM6 were still significantly predictive of overall survival.

Subsequently, the same group of investigators examined whether a gene expression signature correlates with the presence of BRAF mutations in primary cutaneous melanoma [14]. Thus, a cohort of 69 primary melanomas on which global transcript profiles were available was tested for the presence of BRAF mutations. The expression data from these melanomas was analyzed according to BRAF mutational status in 32 melanomas with a BRAF mutation (46%). Two-hundred and nine genes were identified to be differentially expressed in melanomas harboring mutant BRAF, including genes that may be involved in immune responsiveness, such as CD63, MAGE-D2, S100A, and HSP70. In addition, additional genes previously implicated in melanoma progression were identified, including SPP1, CTSB, SERPINE2, and IGFBP2, and in the MITF pathway, such as S100B and MIA-1.

In addition, Alonso et al. [15] examined the gene expression profiles of 34 vertical growth phase melanomas using cDNA microarrays. This tissue set included 21 patients with nodal metastasis vs. 13 without. Supervised analysis of metastasizing melanomas vs. the non-metastasizing group identified 243 differentially expressed genes, of which 206 were upregulated. These included genes involved in numerous cellular pathways, such as cell cycle and apoptosis regulation, epithelial-to-mesenchymal transition, nucleic acid binding, protein synthesis, and metabolism. The prognostic role of SPP1, CDH2, and SPARC on disease-free survival was demonstrated by univariate analysis using immunohistochemical staining.

Table 2.2 Validated molecular prognostic markers for melanoma derived from transcriptomic profiles

Marker	Platform	Outcome parameter	References
RGS1	IHC	RFS, DSS, SLN status	[19, 20]
NCOA3	IHC	RFS, DSS, SLN status	[17, 20]
SPP1	IHC, RNA	RFS, DSS, SLN status	[15, 16, 18, 20]
CDH2	IHC	RFS	[15]
SPARC	IHC	RFS	[15]
MCM4	IHC	OS	[13]
MCM6	IHC	OS	[13]

More recently, Conway et al. [16] performed a profiling analysis of paraffin-embedded primary melanomas using the Illumina DASL array human cancer panel in two distinct cohorts. Intriguingly, SPP1 was identified as the most differentially expressed gene in association with relapse-free survival (RFS) in the first cohort. The association between SPP1 expression and RFS was validated in the second cohort. By multivariate analysis, SPP1 expression level was an independent predictor of RFS when adjusted for age, sex, tumor site, and histological parameters. In addition, the expression of PBX1, BIRC5, and HLF was most strongly correlated with SPP1 expression.

Finally, the prognostic role of several genes identified from the microarray analysis of distinct phases of melanoma progression [5] has been recently demonstrated. From this analysis, three markers (SPP1, NCOA3, and RGS1) were shown to individually predict three outcome parameters for melanoma: sentinel lymph node (SLN) status, RFS, and disease-specific survival (DSS) using immunohistochemical analysis [17–19]. Subsequently, a multi-marker assay incorporating the expression levels of these proteins was examined for its prognostic role in an initial U.S. cohort of 395 patients, and an independent cohort of 141 patients from a distinct population (Germany) [20]. Multi-marker positivity was significantly associated with SLN status and DSS by univariate analysis in the initial cohort. By multivariate analysis, the multi-marker assay remained independently predictive of SLN status and DSS when adjusted for tumor thickness, ulceration, mitotic rate, age, Clark level, gender, and tumor site. In the analysis of DSS, the multi-marker score was the most significant factor predicting DSS, even when SLN status was included as a covariate in the multivariate model. The powerful and independent prognostic impact of the multi-marker assay was separately confirmed using a digital imaging analysis. In the validation cohort, multi-marker positivity was significantly predictive of DSS, and again outperformed routine clinical and histological factors. This is the first demonstration of the prognostic efficacy of a multi-marker assay in melanoma, and the first demonstration of the prognostic efficacy of molecular markers for melanoma in a distinct patient cohort.

Taken together, these results demonstrate the utility of gene expression profiling of primary melanomas, and have identified several novel prognostic markers of melanoma outcome with various degrees of validation (Table 2.2). Intriguingly,

SPP1 has emerged as a common candidate prognostic marker from these profiling studies, whose prognostic impact has now been validated in several distinct cohorts and using different expression platforms.

Gene Expression Profiling of Metastatic Melanoma

Numerous studies of transcriptomic profiling of metastatic melanoma have now been reported. In general, these studies have attempted to either examine various aspects of melanoma biology, or identify potential markers of immune responsiveness. Bittner et al. [1] examined gene signatures of metastatic melanoma using what is now considered an early cDNA microarray platform containing 6,971 unique genes. The authors identified two subtypes of metastatic melanoma, including a dominant cluster, characterized by higher expression of MART-1, CD63, tropomyosin, and WNT5A and reduced expression of fibronectin. This cluster of metastatic melanomas was found to be inversely correlated with uveal melanoma cell lines with increased invasiveness, and showed a trend toward improved survival. Subsequently, Haqq et al. [5] also identified two subtypes of metastatic melanoma, with some overlap in the gene set identified by Bittner et al. The two subtypes were not distinguished by any clinical or histological characteristics or BRAF mutation status, but did differ in that the dominant subgroup exhibited the gene expression signature of vertical growth phase primary melanoma, whereas the second subtype retained the gene signature identified in radial growth phase melanoma. Again, the dominant cluster was associated with an improved prognosis, indicating that radial growth phase metastatic melanoma may be associated with a worse outlook. Together, these studies suggested the prognostic potential of gene expression profiles of metastatic melanoma in small subgroups of patients.

Subsequently, additional studies have extended a number of these observations in larger patient cohorts. John et al. [21] examined genomic profiles of 29 cases of stage III and stage IV melanoma divided into good-prognosis and poor-prognosis subgroups based on time to progression using an oligo-array platform. A predictive score developed using these genes was found to correctly classify nine of ten patients in an independent tissue set, and 12 of 14 patients from a published database. Importantly, this gene signature was developed from a supervised analysis, with no prognostic impact shown for the gene expression profiles.

In addition, Bogunovic et al. [22] examined the combination of gene expression profiles and other markers in the prognostic assessment of metastatic melanoma patients, and identified a group of 266 genes associated with post-recurrence survival. They identified several factors associated with prolonged survival, including mitotic rate of metastatic lesions, an immune response gene signature, and presence of TILs and CD3⁺ cells. Thus gene signatures can be combined with other histological prognostic factors and/or biomarkers to refine the survival associated with metastatic melanoma.

More recently, Jonsson et al. [23] examined the gene expression profiles of 57 patients with stage IV melanoma using the Illumina Beadarray system, and identified four subtypes of metastatic melanoma by unsupervised hierarchical analysis. These four subtypes were grouped as follows: (1) immune response group (characterized by LCK, IFNGR1, HLA class I antigen, CXCL12, and IL1R1 expression); (2) pigmentation differentiation (characterized by high MITF, TYR, SILV, DCT, and low WNT5A levels); (3) proliferation (characterized by high E2F1, BUB1, and CCNA2 expression), and (4) normal-like (characterized by high KRT10 and 17, KIT, FGFR3, and EGFR levels). In addition, BRAF and NRAS mutational analysis and examination of deletions in the CDKN2A gene were performed. Intriguingly, the proliferative subtype was associated with poor survival, high frequency of CDKN2A deletions, and the absence of BRAF or NRAS wild-type samples. In addition, low expression of the gene set associated with immune response signaling was associated with a significantly worse outcome.

Finally, a few studies have used microarray analysis of metastatic melanoma to identify potential signatures of immune responsiveness. Wang et al. [24] analyzed 63 subcutaneous melanoma metastases using a 6,018 cDNA chip in a cohort of patients undergoing various immunotherapies. Global transcript analysis failed to identify subsets of metastases that were predictive of immune responsiveness. However, 30 genes were associated with response to interleukin-2/vaccine-based therapy, including several in the interferon signaling pathway. In addition, Harlin et al. [25] examined gene expression profiles of metastatic melanoma specimens from patients undergoing peptide-based immunotherapy, and identified a major cluster of samples based on the presence or absence of T-cell associated transcripts. Specifically, six chemokines (CCL2, CCL3, CCL4, CCL5, CXCL9, and CXCL10) were confirmed by protein array or qRT-PCR in tumors that contained T cells, with expression of the corresponding receptors observed on human CD8⁺ effector T cells. Finally, functional analysis demonstrated that melanoma cell lines with high chemokine expression recruited human CD8⁺ effector T cells more effectively in xenograft models.

Taken together, transcriptomic profiles of melanoma metastases have resulted in a number of tantalizing observations regarding melanoma biology that still require validation in larger cohorts. Specifically, it will be important to conclusively demonstrate the prognostic impact of gene signatures of melanoma metastases, and to confirm that this is an independent predictor of survival in metastatic melanoma. Since many of the studies have combined specimens from stage III and stage IV patients, the utility of gene expression profiling in distinct subsets of metastatic melanoma patients will need to be demonstrated. It will be important to confirm observations that have suggested divergent survival associated with (1) radial growth phase signature, (2) proliferative signature, and (3) immune responsiveness signature. The ultimate clinical utility of this approach in the setting of metastatic melanoma would require relevance to either (1) identifying high-risk patients for adjuvant therapy trials, (2) identifying patient subsets that may preferentially respond to various systemic therapies, or (3) identifying novel targets for the therapy of metastatic melanoma.

Conclusions

There has been a dramatic increase in the number of genomic analyses of melanoma with the advent of commercially available array platforms and software for bio-statistical analysis. However, these studies are still hampered by the relative paucity of fresh tissue available for analysis, and the lack of coordinated programs for prospective collection of high-quality tissue. While the use of customized array platforms enables the validation of known progression genes, it is still suboptimal for the identification of novel genes in an unbiased manner.

Importantly, however, gene expression profiling efforts in melanoma have enabled novel insights into the biology of melanoma progression. They have resulted in the development of validated multi-marker diagnostic and prognostic markers for primary melanoma. In the setting of metastatic melanoma, further work will be required to conclusively demonstrate the utility of gene expression profiles. In the era of targeted therapy, transcriptomic efforts need to determine relevant gene signatures in the context of the known mutational events in melanoma that can be targeted therapeutically. This may result in the identification of novel pathways for intervention and in the development of rational approaches for combination therapy.

References

1. Bittner M, Meltzer P, Chen Y, et al. Molecular classification of cutaneous malignant melanoma by gene expression profiling. *Nature*. 2000;406:536–40.
2. Clark WH, Elder DE, Guerry IV D, et al. A study of tumor progression: the precursor lesions of superficial spreading and nodular melanoma. *Hum Pathol*. 1984;15:1147–65.
3. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. *Nature*. 2002;417:949–54.
4. Flaherty KT, Puzanov I, Kim KB, et al. Inhibition of mutated, activated BRAF in metastatic melanoma. *N Engl J Med*. 2010;363:809–19.
5. Haqq C, Nosrati M, Sudilovsky D, et al. The gene expression signatures of melanoma progression. *Proc Natl Acad Sci USA*. 2005;102:6092–7.
6. Shoo BA, Sagebiel RW, Kashani-Sabet M. Discordance in the histopathologic diagnosis of melanoma at a melanoma referral center. *J Am Acad Dermatol*. 2010;62:751–6.
7. Kashani-Sabet M, Rangel J, Torabian S, et al. A multi-marker assay to distinguish malignant melanomas from benign nevi. *Proc Natl Acad Sci USA*. 2009;106:6268–72.
8. Talantov D, Mazumder A, Ju JX, et al. Novel genes associated with malignant melanoma but not benign melanocytic lesions. *Clin Cancer Res*. 2005;11:7234–42.
9. Nambiar S, Mirmohammadsadegh A, Hassan A, et al. Identification and functional characterization of ASK/Dbf4, a novel cell survival gene in cutaneous melanoma with prognostic relevance. *Carcinogenesis*. 2007;28:2501–10.
10. Koh SS, Opel ML, Wei JP, et al. Molecular classification of melanomas and nevi using gene expression microarray signatures and formalin-fixed and paraffin-embedded tissue. *Mod Pathol*. 2009;22:538–46.
11. Jaeger J, Koczan D, Thiesen HJ, et al. Gene expression signatures for tumor progression, tumor subtype, and tumor thickness in laser-microdissected melanoma tissues. *Clin Cancer Res*. 2007;13:806–15.

12. Riker AI, Enkemann SA, Fodstad O, et al. The gene expression profiles of primary and metastatic melanoma yields a transition point of tumor progression and metastasis. *BMC Med Genomics*. 2008;1:13.
13. Winnepenninckx V, Lazar V, Michiels S, et al. Gene expression profiling of primary cutaneous melanoma and clinical outcome. *J Natl Cancer Inst*. 2006;98:472–82.
14. Kannengiesser C, Spatz A, Michiels S, et al. Gene expression signature associated with BRAF mutations in human primary cutaneous melanoma. *Mol Oncol*. 2008;1:425–30.
15. Alonso SR, Tracey L, Ortiz P, et al. A high-throughput study in melanoma identifies epithelial-mesenchymal transition as a major determinant of metastasis. *Cancer Res*. 2007;67:3450–60.
16. Conway C, Mitra A, Jewell R, et al. Gene expression profiling of paraffin-embedded primary melanoma using the DASL assay identifies increased osteopontin expression as predictive of reduced relapse-free survival. *Clin Cancer Res*. 2009;15:6939–46.
17. Rangel J, Torabian S, Shaikh L, et al. Prognostic significance of NCOA3 overexpression in primary cutaneous melanoma. *J Clin Oncol*. 2006;24:4565–9.
18. Rangel J, Nosrati M, Torabian S, et al. Osteopontin as a molecular prognostic marker for melanoma. *Cancer*. 2008;112:144–50.
19. Rangel J, Nosrati M, Leong SP, et al. Novel role for RGS1 in melanoma progression. *Am J Surg Pathol*. 2008;32:1207–12.
20. Kashani-Sabet M, Venna S, Nosrati M, et al. A multimarker prognostic assay for primary cutaneous melanoma. *Clin Cancer Res*. 2009;15:6987–92.
21. John T, Black MA, Toro TT, et al. Predicting clinical outcome through molecular profiling in stage III melanoma. *Clin Cancer Res*. 2008;14:5173–80.
22. Bogunovic D, O'Neill DW, Belitskaya-Levy I, et al. Immune profile and mitotic index of metastatic melanoma lesions enhance clinical staging in predicting patient survival. *Proc Natl Acad Sci USA*. 2009;106:20429–34.
23. Jonsson G, Busch C, Knappskog S, et al. Gene expression profiling-based identification of molecular subtypes in stage IV melanomas with different clinical outcome. *Clin Cancer Res*. 2010;16:3356–67.
24. Wang E, Miller LD, Ohnmacht GA, et al. Prospective molecular profiling of melanoma metastases suggests classifiers of immune responsiveness. *Cancer Res*. 2002;62:3581–6.
25. Harlin H, Meng Y, Peterson AC, et al. Chemokine expression in melanoma metastases associated with CD8+ T-cell recruitment. *Cancer Res*. 2009;69:3077–85.



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