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TOPICAL COLLECTION ON SURGICAL ONCOLOGY (A TUFARO, SECTION EDITOR)

Gene Signatures in Cutaneous Malignancies

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Abstract

Purpose of Review In this review, we will discuss the recent advances in the identification of landmark gene signatures in cutaneous melanoma and in the discovery of those relevant to cutaneous squamous cell carcinoma (cSCC).

Recent Findings Melanoma and cSCC are the most important cutaneous malignancies when considering morbidity and mortality. They are responsible for the greatest number of skin cancer related deaths. Over the past several years, a number of gene signatures have been identified showing great promise in terms of tumor molecular classification and risk stratification of patients to anticipate best therapeutic modalities. These gene signatures have allowed a personalized medicine approach to a comprehensive decision-making process for these patients.

Summary Prediction of the prognosis and therapeutic response of patients with melanoma and high-risk cSCC will be aided by the elucidation and utilization of these gene signatures.

Keywords Gene signature · Cutaneous melanoma · Cutaneous squamous cell carcinoma

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Introduction

Many gene signatures, such as MammaPrint (Agendia, Inc.) in breast cancer [1••] and DecisionDx-Melanoma (Castle Biosciences Inc.) in cutaneous melanoma [2••], have been the underpinning of personalized medicine. A gene signature is defined as a single or a combined genetic alteration with validated specificity in terms of diagnosis, prognosis, or prediction of therapeutic response. This specificity should be validated in independent groups of tumors and, if possible, by different techniques and teams [3••]. There are three key points needed to define a gene signature: (1) select and identify a gene signature in a training data set; (2) validate the gene signature in an independent validation data or test set; (3) establish clinical trials to validate the gene signature in a clinical setting to transfer it to daily clinic practice.

Essentially, gene signature is a gene expression alteration, which is usually identified and characterized by the following steps: (i) select two groups of samples (tumor vs. normal or treated vs. untreated), producing a training data set; (ii) compare the two groups of samples in the training data set, identify differentially expressed genes, select the most upregulated or downregulated genes that are specific to a disease condition (tumor) or response (treatment), establish a model and scaling coefficient, or perform survival analysis according to selected model; (iii) select an independent group of samples (tumor and normal or treated and untreated), producing an independent validation data set; (iv) split the samples in the validation data set according to the gene signature (strictly as determined in the training data set) and track outcomes or survival analysis. Then a clinical trial can be done to treat patients based on a gene signature score, then outcomes or survival analysis can be tracked to clinically validate the gene

signature classification and transfer it to daily clinic practice [3••].

The advent of two unique techniques in 1995 critically contributed to the initialization of analysis and identification of gene signatures for physiological or clinical relevance. The first technique is Serial Analysis of Gene Expression (SAGE) which improves expressed sequence tag (EST) analysis by allowing simultaneously quantitative analysis of a large number of transcripts in a sample to demonstrate more easily characteristic gene expression patterns [4]. The second technique is DNA microarray which quantifies complementary DNA (cDNA) hybridization on a glass slide to analyze the expression of thousands of genes in parallel [5].

DNA microarray is a widely adopted technique to profile gene expression signatures to best classify the tumor subtypes [6] and to predict patient outcomes [7, 8] and response to therapy [9, 10]. The most successful gene signature developed by far is the breast cancer 70-gene signature (MammaPrint), which was the first in vitro diagnostic multivariate index assays (IVDMIA) cleared in 2007 by the Food and Drug Administration (FDA): Mammaprint was the only molecular diagnostic test with a randomized prospective clinical trial validating clinical utility. This 70-gene signature could distinguish patients at a significant risk for distant relapse and death from those at low risk, thus improve prediction of clinical outcomes in women with early-stage breast cancer. Also it could add an independent prognostic value in selecting patients for adjuvant chemotherapy when combined with the standard clinical-pathological criteria [1••, 9–11]. A diagram illustrating the process of gene signature identification and characterization is shown in Fig. 1.

Cutaneous malignancies arise from keratinocytes, melanocytes, Merkel cells, endothelial cells, adnexal structures, constituents of the connective tissue stroma, and skin-resident immune cells among others. Cutaneous melanoma and cutaneous squamous cell carcinoma (cSCC) are the most important tumors in cutaneous malignancies. Cutaneous melanoma is the third most common cutaneous malignancy after basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) and is the leading cause of death from skin cancer. In 2018, it is estimated that 91,270 cases of melanoma are diagnosed and 9320 deaths are expected in the USA [12••, 13]. Cutaneous SCC is the second most common non-melanoma skin cancer (NMSC), with over 700,000 new cases diagnosed annually resulting in 3932–8791 deaths in the USA in 2012. Cutaneous SCC is the most common skin cancer in transplant patients and has a 60–250-fold increased incidence in solid organ transplant recipients (OTRs) compared to immunocompetent individuals. Skin cancer is also the most frequent malignancy with SCC and BCC accounting for 95% malignancies with a 4:1 SCC to BCC ratio [14•, 15, 16].

We have searched the PubMed with the keywords "gene signature" plus "cutaneous malignancy" and found that most of the publications available are involved with gene signatures in cutaneous melanoma. Due to the importance of cSCC in cutaneous malignancies, the current review will put major focus on the gene signatures in cutaneous melanoma and cSCC, their implications in terms of molecular classification, and predicting the prognosis and therapeutic response of patients with these malignancies.

Molecular Classification Gene Signature for Cutaneous Malignancies

In 2000, Bittner et al. [17] published the first evidence showing that classification of melanoma on the basis of gene expression profile is possible, which led to the numerous 2 decades of studies on gene signatures in cutaneous melanoma and cSCC. Since then, several gene signatures have been identified for molecular classification of cutaneous melanoma and cSCC. The major advances in molecular classification of cutaneous melanoma and cSCC are summarized in Table 1 and discussed below.

Gene Signature Associated with Cell Phenotypes

In 2006, Hoek et al. [18] identified two transcription signatures (proliferative and invasive signatures) by carrying out three separate DNA microarray analyses on a total of 86 melanocyte and melanoma cell cultures, which classify them into proliferative and invasive cell phenotypes upon a switch in melanoma progression. A *proliferative* signature represented weakly metastatic melanomas, susceptible to the transforming growth factor β (TGFβ)-mediated inhibition of proliferation with a low motility. An *invasive* signature represented strongly metastatic melanomas, resistant to TGFβ and highly mobile.

Gene Signature Associated with BRAF Mutation Status

In 2008, Kannengiesser et al. [19] reported a 209-gene signature which were significantly associated with BRAF mutation status (raw $P \leq 0.001$). This gene signature was identified by analyzing the expression data obtained after hybridization on a whole genome 44K oligonucleotide microarray (Agilent) for 69 patient samples including 32 melanomas with BRAF mutation and 37 wild type (WT)

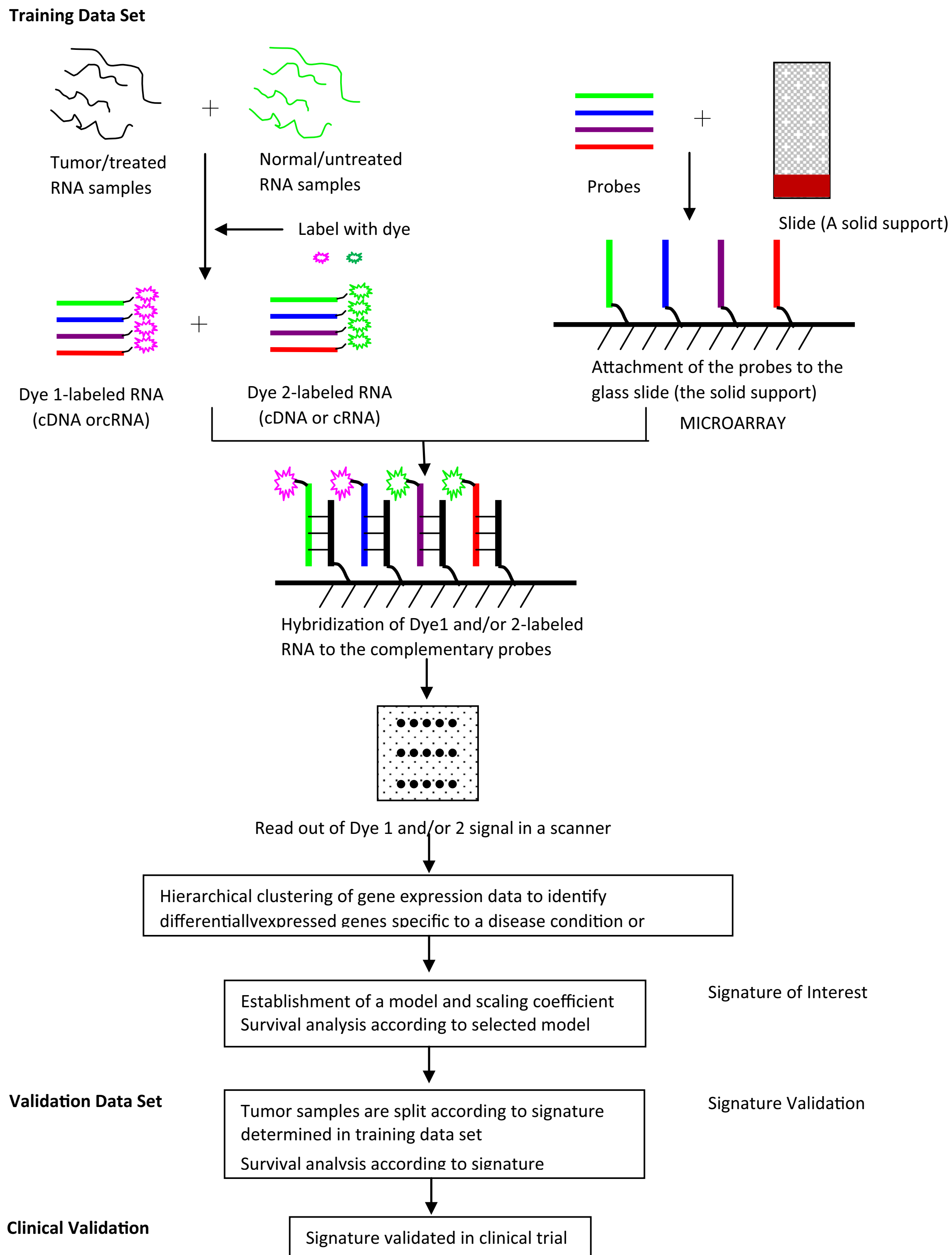

Fig. 1 Gene signature identification and characterization. Gene signature is identified and characterized through three steps: signature identification (training data set), signature validation (validation data set), and finally the clinical validation

melanomas. Among the genes that differentiated most strongly between BRAF mutated and non-mutated melanomas, there were those involved in *melanoma immune responses* such as MAGE-D2, CD63, and HSP70.

Table 1 Molecular classification gene signature identification and assessment studies in cutaneous melanoma and cSCC

Signature	Gene expression assay	Tissue source	Training set size and sample type	Independent validation set size and tissue type	Classification significance and performance		Application	References	Year
					Training set	Validation set			
Gene expression pattern	cDNA array	Fresh	31 melanomas and 7 controls	N/A	31 melanomas partitioned into two groups of 12 and 19	N/A	The major cluster of 19 melanomas having reduced motility, invasive ability and vasculogenic mimicry	Bittner [17]	2000
Two transcription signatures	Affymetrix array	N/A	86 melanocyte and melanoma cell cultures	N/A	N/A	N/A	Classifying melanoma cells into proliferative and invasive phenotypes, involving in cell phenotype switch	Hoek [18]	2006
209-gene signature	Agilent array	Fresh	69 melanomas including 32 BRAF and 37 wild type melanomas	N/A	Associated with BRAF mutation Raw $P \leq 0.001$	N/A	Discrimination of BRAF from wild type in melanomas	Kannengiesser [19]	2008
23-gene signature	qRT-PCR	FFPE	254 melanomas and 210 nevi	211 melanomas and 226 nevi	Sensitivity: 89% Specificity: 93%	Sensitivity: 90% (95% CI 85–93%). Specificity: 91% (95% CI 87–95%)	Differentiating benign nevi from malignant melanomas	Clarke [20]	2015
	qRT-PCR	FFPE	N/A	736 triple-concordant lesions, including 559 benign and 177 malignant	N/A	Sensitivity: 91.5% (95% CI 86.4–95.2%) Specificity: 92.5% (95% CI 90.0–94.5%)	Assessment	Clarke [21]	2017
	qRT-PCR	FFPE	N/A	182 lesions with 99 stage I, II, or III malignant melanomas and 83 benign nevi	N/A	Sensitivity: 93.9% Specificity: 96.2%	Assessment	Ko [22]	2017
17-gene profile	Affymetrix array	Fresh	8 cSCC, 8 site matched non-tumor-bearing, 8 psoriasis, and 5 non-lesional skin biopsies	7 cSCC, 7 site matched non-tumor-bearing, 7 psoriasis, and 7 non-lesional skin biopsies	N/A	N/A	A cSCC-specific gene expression profile and a profile to distinguish malignant hyperproliferation from benign hyperplasia identified	Haider [23]	2006

Table 1 continued

Signature	Gene expression assay	Tissue source	Training set size and sample type	Independent validation set size and tissue type	Classification significance and performance		Application	References	Year
					Training set	Validation set			
MMP1	Affymetrix array	Fresh FFPE	12 fresh tissue samples (6 cSCC and 6 matching normal skin)	(i) qRT-PCR: 27 FFPE samples (22 tumors and 5 normal skin) and 69 fresh tissues (32 tumors and 37 normal skin) (ii) IHC: 122 FFPE samples (47 non-aggressive and 75 aggressive)	164 differentially expressed genes identified	High expression associated with aggressive tumors (OR 1.01; 95% CI 1–1.03; $P = 0.034$ for mRNA. OR 5.47; 95% CI 0.73–2.68; $P < 0.001$ for protein) The sensitivity and specificity of MMP1 were 82% and 62% for mRNA and 45% and 87% for protein	Discriminating between aggressive vs non-aggressive cSCC tumors	Prasad [14•]	2014

cSCC cutaneous squamous cell carcinoma, *IHC* immunohistochemistry, *qRT-PCR* quantitative reverse transcription PCR, *FFPE* formalin-fixed paraffin-embedded, *triple-concordant lesion* a lesion with a concordant pathological diagnosis assigned by 3 experienced dermatopathologists, *OR* odds ratio, *CI* confidence interval, *N/A* not available/not applicable

Gene Signature to Differentiate Benign and Malignant Melanocytic Neoplasms

In 2015, Clarke et al. [20] described a 23-gene expression signature that effectively differentiated benign and malignant melanocytic neoplasms. This gene signature was identified by qRT-PCR analyzing RNA expression of a training set of 464 FFPE (formalin-fixed paraffin-embedded) samples including 254 melanomas (with broad clinical spectrum-superficial spreading, nodular, acral, lentigo maligna/lentigo maligna melanoma, and other) and 210 nevi (compound, junctional, intradermal, spitz, blue, and other), which was validated with a test data set of 437 FFPE samples including 211 melanomas (superficial spreading, nodular, acral, lentigo maligna/lentigo maligna melanoma, and other) and 226 nevi (compound, junctional, intradermal, spitz, blue, and other). The signature test sensitivity and specificity were 90% (95% CI 85–93%) and 91% (95% CI 87–95%) in the validation set.

In 2017, Clarke et al. [21] assessed the association between the 23-gene signature score and the pathologic diagnosis, using a validation set of 736 triple-concordant FFPE samples selected from 1400 melanocytic lesions. To ensure pathologic diagnostic accuracy, a triple-concordant diagnosis was required, meaning a clinically relevant sample needed diagnostic concordance determined by 3 experienced dermatopathologists before inclusion in the validation set. This validation set consisted of 177 malignant lesions (acral melanoma, lentigo maligna/lentigo maligna melanoma, nodular melanoma, and superficial spreading melanoma and others, which were similar to the subtypes of Clarke et al. at 2015 without desmoplastic melanoma) and 559 benign melanocytic nevi (a wide range of subtypes). The expression of the 23-gene signature was measured by qRT-PCR assay for each FFPE tissue sample, which was converted to a signature score by a weighting algorithm to classify benign and malignant lesions. The signature test had a sensitivity of 91.5% (95% CI 86.4–95.2%) and a specificity of 92.5% (95% CI 90.0–94.5%), which showed that the signature has a high ability to differentiate benign nevi from malignant melanoma in a diverse array of samples encountered in routine clinical practice.

Ko et al. [22] validated the 23-gene signature using qRT-PCR with a cohort of 182 archival FFPE cases, which included 99 malignant lesions (12 subtypes) and 83 benign melanocytic nevi (18 subtypes). The malignant lesions in this cohort were stage I, II, or III primary cutaneous melanomas that produced distant metastases. In this validation, the signature test had a sensitivity of 93.9% and a specificity of 96.2%, showing once again that the signature had a high diagnostic accuracy to differentiate malignant melanoma from benign nevi.

Gene Signature to Distinguish Malignant Hyperproliferation of cSCC from Benign Hyperplasia

In 2006, Haider et al. [23] was the first to publish specific gene expression patterns that define a profile for primary cSCC and distinguish malignant hyperproliferation (cSCC) from benign hyperplasia (Psoriasis vulgaris) by using a hierarchical clustering approach. This early study analyzed mRNA expression from eight cSCC specimens, eight site matched non-tumor-bearing (N) specimens, eight psoriasis (P) specimens, and five non-lesional (NL) skin biopsies by gene array (HG-U95A/Av2 chips, Affymetrix). By hierarchical clustering of the RNA expression results, a cSCC-specific gene expression profile was identified, in which HPGD and FZD6 expression were increased in cSCC alone. They also identified a gene expression profile to distinguish malignant hyperproliferation from benign hyperplasia, in which hyperproliferation was characterized by upregulation of MMP1, 10, and 13, CTSL2, CST6, STAT3, MSMB and downregulation of iNOS, CD83, CD8a, GZMB, and the hyperplasia was associated with upregulation of DEFB4, SERPINB3, STAT1, K16, CEACAMs, and WNT 5A. The gene expression profiles identified were validated by qRT-PCR for mRNA from cSCC, N, P, and NL skin biopsy specimens ($n = 7$ for each). This early study was limited by its sample size ($n \leq 8$ for each). Nevertheless, it was the first report to suggest that a gene expression signature may identify cSCC tumors and distinguish between malignancy and benignancy of a tumor that could translate into clinical therapeutic implications.

Gene Signature to Distinguish Between Aggressive and Non-aggressive cSCC Tumors

In 2014, our lab found that MMP1 could be used as a gene signature to discriminate between aggressive and non-aggressive cSCC tumors by using a combination of microarray, qRT-PCR, and immunohistochemistry to examine 200 skin samples [14•]. In our study, 164 differentially expressed genes were first identified by using Affymetrix HGU133 2.0 Plus GeneChip from 12 fresh tissue samples (6 cSCC and 6 matching normal skin). Of the 164 genes identified, 12 genes were selected and validated by qRT-PCR in a separate set of 27 paraffin-preserved samples (22 tumors and 5 normal skins). Of the 12 genes validated, three genes (MMP1, MMP10, and ADAMTS1) were further validated by qRT-PCR in an additional set of 69 fresh tissue samples (32 tumors and 37 normal skin) for mRNA expression and validated by immunohistochemistry in 131 paraffin-preserved tissue sections (80 arrayed and 51 non-arrayed samples) and 9 normal skin samples for the protein expression. Univariate analysis on the mRNA expression of the three genes in 32 fresh samples (16 aggressive vs. 16 non-aggressive) and the protein expression of the three genes in 122 paraffin-preserved samples (75 aggressive vs. 47 non-aggressive) showed that only MMP1 was significantly highly expressed in aggressive tumors compared with non-aggressive tumors (OR 1.01; 95% CI 1–1.03; $P = 0.034$ for mRNA expression. OR 5.47; 95% CI 0.73–2.68; $P < 0.001$ for protein expression). The sensitivity and specificity of MMP1 to discriminate between aggressive and non-aggressive tumors were 82% and 62% for mRNA expression and 45% and 87% for protein expression.

Gene Signatures with Prognostic Relevance

In 2006, Winnepenninckx et al. [24] were the first to publish gene signatures with prognostic relevance in melanoma. This early study identified a 254-gene signature associated with 4-year distant metastasis-free survival (DMFS) by class comparison analysis of gene expression data from 58 patients with primary melanomas. Since the initial search for prognostic signatures by Winnepenninckx, several prognostic gene signatures have been identified in cutaneous melanoma. The major advances in gene signatures with prognostic relevance in cutaneous melanoma are summarized in Table 2.

Gerami et al. [31••] advanced the use of gene signature prognostic studies in cutaneous melanoma by identifying 28 class-discriminating gene targets (AQP3, ARG1, BAP1 5′ region, BAP1 3′ region, BTG1, CLCA2, CRABP2, CST6, CXCL14, DSC1, EIF1B, GJA1, ID2, KRT14, KRT6B, LTA4H, MGP, PPL, RBM23, ROBO1, S100A8, S100A9, SAP130, SPP1, SPRR1B, TACSTD2, TRIM29, TYRP1) associated with the metastatic risk of cutaneous melanoma, with later inclusion of 3 endogenous control genes producing a 31-gene signature. As an ancillary tool, when this gene signature was combined with the AJCC staging system, it identified 80% (24/30) of stage I and IIA cases and 70% of sentinel lymph node (SLN)-negative patients who eventually developed metastasis and 5.3% of thin tumor patients (2.0% of T1a and 13.9% of T1b) who eventually developed recurrence and distant metastasis [12••, 31••]. These cases, however, would not have been able to be identified by the AJCC staging system. This gene signature has been successfully developed to a commercially available test for cutaneous melanoma, known as DecisionDx-Melanoma, by Castle Biosciences [2••].

Table 2 Prognostic gene signature identification and assessment studies in cutaneous melanoma

Signature	Gene expression assay	Sample size and AJCC stage		End point and prognostic significance (P value or HR)		Predictive prognosis	References	Year
		Training set	Validation set	Training set	Validation set			
254-GS	Agilent array	83 primary melanomas	17 + 238 primary melanomas	254 genes associated with DMFS	MCM4 and MCM6 associated with OS	Outcome	Winnepenninckx [24]	2006
PLZF gene	ABI array	41 stage IB-IV primary melanomas, 29 metastases, 10 normal skin, 6 cell lines	N/A	Associated with OS. $P < 0.05$	N/A	Malignant melanoma survival risk (OS)	Brunner [25]	2008
9-GS	qRT-PCR	91 stage IA-IIIC primary CM	44 stage IA-IIIB primary CM	Associated with OS. $P < 0.0001$. HR = 3.83	Validated with 44 CM.	Outcome (OS, DMFS)	Brunner [26]	2013
53-GS	NanoString nCounter	40 stage II–III primary CM. Public data from 46 primary CM (GSE15605)	48 stage II–III primary melanomas	Associated with non-progression, DSS and RFS. $P < 0.001$	Associated with non-progression, RFS, and DSS. $P < 0.001$, 0.001 or = 0.024	Outcome (non-progression, RFS and DSS)	Sivendran [27]	2014
192-GS	Affymetrix array, Illumina beadchip	Two public cell line xenograft datasets (GSE7929 and GSE7956)	Two public melanoma datasets (GSE8401 and GSE19234)	192 genes identified by BRB-ArrayTools	High-risk group associated with shorter OS. $P < 0.01$ and $P = 0.02$ in metastatic tumor group and subgroup	Metastatic melanoma patient survival (OS)	Chen [28]	2014
12-GS	Affymetrix array	120 stage IV non-locoregional metastatic melanomas	10 stage IV non-locoregional metastatic melanomas	Defined a GS score, classifying patients into the highest and lowest score groups	Correlated with the presence of TL-ELNS, which associated with better OS. $P = 0.008$	Presence of TL-ELNS, associated with better OS	Coppola [29] Messina [30]	2011 2012

Table 2 continued

Signature	Gene expression assay	Sample size and AJCC stage		End point and prognostic significance (P value or HR)		Predictive prognosis	References	Year
		Training set	Validation set	Training set	Validation set			
31-GS	qRT-PCR	164 stage 0–IV CM	104 stage I–IV CM	Associated with DFS. $P < 0.0001$	Associated with DFS. $P < 0.0001$	Metastatic risk of primary CM (DFS)	Gerami [31••]	2015
		N/A	217 stage I–IV CM	N/A	Associated with DFS, DMFS and OS. $P < 0.0001$	Metastatic risk in SLNB eligible patients (DFS, DMFS and OS).	Gerami [32]	2015
		N/A	690 stage I–III CM	N/A	Associated with RFS, DMFS, and MSS. $P < 0.0001$, 0.01, 0.001 or = 0.007	Identify high-risk patients from those with low-risk SLN (−), stage I–IA and ≤ 1 mm T1 thin tumors	Gastman [12••]	2019
		N/A	523 stage I–III CM	N/A	Associated with RFS, DMFS. $P < 0.001$ or 0.01	Metastatic risk (RFS, DMFS)	Zager [33]	2018
4-class structure GS	Illumina Beadchip	57 stage IV distant metastatic melanomas	20 stage IV liver metastatic melanomas	OS differed significantly between four subtypes (P = 0.01), with the proliferative subtype associated with the shortest OS ($P = 0.003$)	Two long-term survivors were in the high immune response group	Outcome (OS)	Jonsson [34]	2010
		223 primary melanomas, including 205 CM and 18 acral lentiginous melanomas and mucosal melanomas	2 independent public datasets	Converged to high and low-grade groups. High-grade group associated with poorer RFS (HR 4.94; 95% CI 2.84–8.59) and OS (HR 3.66; 95% CI 2.40–5.58)	High-grade group associated with poorer outcomes in stage III melanomas ($P = 0.003$) and primary melanomas ($P = 0.04$)	Outcome (RFS, OS)	Harbst [35]	2012
		214 malignant CM	309 regional and distant metastatic lesions from the TCGA dataset	In the patients with regional metastases, DMFS in pigmentation and proliferative groups and DSS in proliferative group were increased compared to those in high immune response group. $P = 0.03$, 0.003 and 0.002	DSS in high immune response group was improved compared to other groups. $P = 5 \times 10^{-4}$	Outcome (DMFS, DSS)	Cirenajwis [36]	2015

Table 2 continued

Signature	Gene expression assay	Sample size and AJCC stage		End point and prognostic significance (P value or HR)		Predictive prognosis	References	Year
		Training set	Validation set	Training set	Validation set			
4-lncRNA GS	RNA-seq, Illumina Beadchip	235 CM from TCGA dataset	223 and 210 CM from TCGA and GSE65904 datasets	Associated with OS $P < 0.001$	Associated with OS $P = 0.002$ for TCGA and $P < 0.001$ for GSE65904	Duration of survival of patients (OS)	Chen [37]	2017

GS gene signature, *CM* cutaneous melanoma, *HR* hazard ratio, *CI* confidence interval, *OS* overall survival, *DMFS* distant metastasis-free survival; DFS, disease-free survival, *DSS* disease-specific survival, *MSS* melanoma-specific survival, *RFS* recurrence-free survival, *TL-ELNS* tumor-localized ectopic lymph node-like structures, *SLN* sentinel lymph node, *SLNB* sentinel lymph node biopsy, *lncRNAs* long non-coding RNAs, *N/A* not available/not applicable

Gene Signatures with Predictive Relevance

In 2013, Ulloa-Montoya et al. [38] reported a 84-gene signature associated with the clinical response for MAGE-A3 immunotherapeutics in two phase-II trials comparing the recombinant MAGE-A3 protein combined with immunostimulants (AS15 and AS02B). This gene signature was identified by use of Affymetrix HG-U133 Plus 2.0 microarray with qRT-PCR from a training set of 56 patients with unresectable MAGE-A3—positive stage III or IV M1a metastatic melanoma. Overall Survival (OS) was notably greater in the population of melanoma patients whose tumor had the gene signature. The median OS was 16.2 months (95% CI 9.0 to 20.0 months) in the signature-negative population and 29.0 months (95% CI 20.5 to 40.2 months) in the signature-positive population. This effect was strongest when the immunostimulant AS15 was included in the immunotherapy. The OS was 16.2 months [95% CI 4.5 months to not reached (NR)] for signature-negative patients and 53.7 months (95% CI 29.0 months to NR) for signature-positive patients among the AS15-treated patients. The hazard ratio (HRs) for OS between the signature (+) and (–) populations was 0.37 (95% CI 0.13 to 1.05; $P = 0.06$) in the patients treated with MAGE-A3 + AS15. When the same gene signature was used to predict the outcome of the patients who were treated with MAGE-A3 plus AS02B in a validation set of 157 patients with completely resected MAGE-A3–positive non-small-cell lung cancer [NSCLC] (stage IB/II), actively treated signature (+) patients showed a favorable disease-free interval (DFI) compared to placebo-treated signature (+) patients (HR 0.42; 95% CI 0.17 to 1.03; $P = 0.06$). Further clinical trials (phase II and III) did not show that this 84-gene signature could be predictive when applied to metastatic melanoma following MAGE-A3 immunotherapy [39, 40] (Table 3).

Conclusion

Metastatic melanoma is one of the most aggressive and therapy resistant human cancers, and, in 2011, the 5-year relative survival was only 16% [41]. The current treatment strategies used for metastatic melanoma include surgery, immunotherapy, targeted therapy, radiation therapy, and chemotherapy. Several systemic therapies have been shown to improve recurrence-free survival (RFS) in the patients with high-risk, resected, stage IIB-IIIC melanoma, [42–45] or unresectable stages III and IV melanoma [44–46]. The decision to select the type of adjuvant therapy after surgery or the type of systemic therapy for an

Table 3 Predictive gene signature identification and assessment studies in cutaneous melanoma

Signature	Gene expression assay	Tissue source	Training set size and tumor stage	Independent validation set size and tumor stage	End point and predictive significance (P value)		Clinical trial	Predictability	References	Year
					Training set	Validation set				
84-gene expression signature	Affymetrix array	Fresh	56 nonresectable MAGE-A3–positive stage III or IV M1a metastatic melanoma	157 completely resected MAGE-A3–stage IB/II non–small-cell lung cancer	Associated with OS HR 0.37 (95% CI 0.13–1.05, P = 0.06)	Associated with DFI HR 0.42 (95% CI 0.17–1.03, P = 0.06)	Phase II	A predictor associated with clinical response to MAGE-A3 immunotherapy	Ulloa-Montoya [38]	2013
	Affymetrix array	Fresh	N/A	123 unresectable, MAGE-A3-positive stage IIIB-C or IV-M1a cutaneous melanoma	N/A	Not associated with OS, PFS, and MTTF	Phase II	Not predictive of clinical outcome for MAGE-A3 immunotherapy	Saiag [39]	2016
	qRT-PCR	FFPE	366 tumor invaded lymph nodes (LN) samples	729 tumor invaded lymph nodes (LN) samples	55 genes of 84-GS measured, leading to a 39-GS identified, which associated with a clinical benefit for MAGE-A3 immunotherapy (HR for DFS = 0.61, P = 0.03).	39-GS not associated with MAGE-A3 treatment in terms of DFS, OS, DFSS and DMFS	Phase III	Not predictive of clinical response to MAGE-A3 immunotherapy	Dreno [40]	2018
4-class structure gene signature	Illumina BeadChip	Fresh	214 Cutaneous malignant melanoma	Three external datasets from melanoma patients receiving targeted therapy or MAGE-A3 immunotherapy (GSE50509; GSE61992; GSE35640)	In the patients with regional metastases, DMFS in pigmentation and proliferative groups and DSS in proliferative group were increased compared to those in high immune response group. P = 0.03, 0.003 and 0.002	No clear association with the signature to predict the response to therapies	N/A	Not predictive of clinical response to therapies	Cirenajwis [36]	2015

GS gene signature, *HR* hazard ratio, *CI* confidence interval, *OS* overall survival, *DFI* disease-free interval, *PFS* progression-free survival, *MTTF* median time-to-treatment failure, *DFS* disease-free survival, *DFSS* disease-free-specific survival, *DMFS* distant metastasis-free survival, *DSS* disease-specific survival, *N/A* not available/not applicable

individual melanoma patient is based on the relative risk of recurrence and death of the patient.

Treatment modality, for the most part, is determined by the AJCC staging system. Currently, the most important prognostic predictors for melanoma proposed by AJCC are the Breslow depth, the ulceration, sentinel lymph node biopsy (SLNB) result, the number of positive lymph node involvement, and the presence or absence of distant metastasis (the 8th Edition) [47]. However, the AJCC staging system does not cover every aspect of melanoma. For example, Gastman et al. [12••] reported that the melanoma-specific survival (MSS) rates for stage I, II, and III patients in a pooled cohort of 690 patients from the prior studies diagnosed between 1998 and 2014 were similar to those in the AJCC 8th Edition International Melanoma database (with a difference of $\pm$ 1% for the MSS rates between the pooled cohort and the AJCC 8th Edition database), indicating that the 690 patient cohort was representative of contemporary patients with melanoma in terms of staging by the AJCC 8th Edition. Within this 690 patient cohort, a proportion of patients with node negative, stage I–IIA ,and T1 ($\leq$ 1 mm) melanoma were found to have a significant high risk of recurrence, distant metastasis, and death, which however was deemed as the low-risk type of patients in the prior edition of AJCC system.

The complexity of clinical presentation, the difficulty of pathological diagnosis, the dependency of treatment options on the pathological diagnosis and the unpredictability of therapeutic response urge the advent of novel tools in the management of the patients with cutaneous malignancies. As a new technique, using a gene signature has created a great interest in tumor molecular classification and the prediction of patients' prognosis and therapeutic response. In molecular classification, a gene signature can be used to classify melanoma into different phenotypes, to predict melanoma BRAF mutation status, to distinguish malignant from benign nevi, to distinguish malignant cSCC hyperproliferation from benign hyperplasia, and to predict aggressive cSCC tumors from non-aggressive cSCC tumors. Gene signatures have been shown to predict metastatic risk of malignant melanoma (DFS, DMFS, RFS, and OS), clinical outcome (OS, DMFS, non-progression, RFS, and DSS), the presence of tumor-localized ectopic lymph node-like structures (TL-ELNS) of melanoma and identify the high-risk patients from those with AJCC low-risk SLN (-), stage I-IA, or $\leq$ 1 mm T1 thin tumors (RFS, DMFS, and MSS). These findings are critical in risk stratifying melanoma patients. A gene signature has the potential in personalized medicine to be used to predict clinical response for therapeutic interventions.

Regardless of the success of Castle's gene signature assay (DecisionDx-Melanoma) in determining outcomes, most of the gene signatures identified have not been assessed by a clinical trial in a clinical setting. Although phase II and III clinical trials were performed for an 84-gene signature (GS) to predict clinical responses to MAGE-A3 immunotherapeutics combined with immunostimulants (AS15 and AS02B), the two trials failed. The GS-positive and GS-negative cutaneous melanoma patient populations did not differ between the MAGE-A3 and placebo groups in terms of disease-free survival, overall survival, disease-free-specific survival, or distant metastasis-free survival in any of the analyses or in the assessment of disease-free survival for each year of follow-up [39, 40]. In cSCC, to our knowledge, there are no studies published to date on gene expression signature except Haider AS report [23] and our study [14•] on molecular classification of cSCC. More extensive studies are needed to explore the gene signatures for molecular classification, prognosis, and therapeutic response prediction in cutaneous melanoma and cSCC, especially cSCC, in the future to benefit the tumor patients.

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- •• Of major importance

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