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Original contribution

Correlation of melanoma gene expression score with clinical outcomes on a series of melanocytic lesions☆,☆☆

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Summary A 23-gene expression signature was recently developed as an adjunct to histopathology to differentiate melanocytic nevi from melanoma. The current study correlated the gene expression signature scores to actual clinical outcomes in cases from the first validation study. RNA was extracted from 127 archival formalin-fixed paraffin-embedded tissue sections of melanocytic lesions. Gene expression was measured using quantitative reverse-transcription polymerase chain reaction, and a weighting algorithm was used to generate a numeric score. Gene expression test results were compared to histopathological diagnoses and development of local recurrence, sentinel lymph node metastases, and distant metastases. Sixty-five lesions were diagnosed histopathologically as melanoma. Fourteen developed metastases. Gene expression test results were malignant in 61 of 65 (93.8%) lesions (including all lesions that metastasized), indeterminate in 2 of 65 (3.1%) lesions, and benign in 2 of 65 (3.1%) lesions. The remaining 62 lesions were diagnosed as benign by histopathology. Gene expression test results were benign in 48 of 62 (77.4%), indeterminate in 7 of 62 (11.3%), and malignant in 7 of 62 (11.3%). There was a strong correlation between the gene expression signature test results and clinical outcomes. All lesions that metastasized were correctly identified by the test as malignant melanoma.

1. Introduction

Although the incidence of melanoma in the United States continues to rise [1], many melanomas are curable if detected early. Ten-year survival rates range from 86% to 95% for stage I melanoma compared to only 10% to 15% for stage IV [1]. The early and accurate diagnosis of melanoma is therefore critical to patient outcomes. The current standard for diagnosis is histopathologic evaluation, but some melanocytic neoplasms are difficult to

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Table 1 Summary of clinical and histologic data by disease status

Variable	Summary statistic	Disease status		
		Metastatic melanoma (n = 14)	Melanoma (n = 51)	Benign nevi (n = 62)
Age	Mean (SD)	58 (18)	66 (16)	42 (17)
Sex	Male (%)	10 (71)	35 (69)	24 (39)
	Female (%)	4 (29)	16 (31)	38 (61)
Breslow depth	Median (range)	1.76 (1.02-9.00)	1.25 (0.58-9.00)	NA
	0-1.0 mm (%)	0 (0)	17 (35)	NA
	1.01-2.0 mm (%)	7 (50)	19 (39)	NA
	>2.0 mm (%)	7 (50)	13 (27)	NA
	Missing	0	2	NA
Ulceration status	Present (%)	7 (50)	9 (18)	NA
	Absent (%)	7 (50)	40 (82)	NA
	Missing	0	2	NA
Length of follow-up (mo.)	Median (range)	14 (0-58)	48 (0-95)	30 (0-72)

Abbreviation: NA, not applicable.

classify by histopathology alone, and even experts occasionally disagree on diagnoses [2-6]. As such, even a consensus diagnosis by experts may not correlate with the ultimate clinical behavior of histopathologically ambiguous melanocytic neoplasms [7,8].

In response to these diagnostic challenges, ancillary tests have been developed to aid in the differentiation of ambiguous melanocytic lesions. Such methods include the detection of gene copy number aberrations (array comparative genomic hybridization and fluorescence in situ hybridization [FISH]) as well as differential gene expression assays (a 23-gene expression signature). These methods have been shown to differentiate melanoma from nevi with sensitivities of 92% to 95% for array comparative genomic hybridization [9,10] and 90% to 92% for the 23-gene signature [11,12]. Reported sensitivity of the FISH method varies from 43% to 94% depending upon the lesion subtype, the extent of histopathologic correlation, and the particular assay specifications (probe set and threshold values) [10,13-17].

Table 2 Results of gene expression testing by histopathologic subtype

	Total	Gene expression test result		
		Malignant	Indeterminate	Benign
Melanoma Subtype				
Acral lentiginous and spindle cell melanoma	1	1	0	0
Acral lentiginous melanoma	4	4	0	0
Acral lentiginous melanoma w/ dominant nodular component	1	1	0	0
Acral melanoma, NOS	2	2	0	0
Desmoplastic melanoma	2	0	2	0
Lentigo maligna melanoma	8	8	0	0
Nodular melanoma	18	18	0	0
Superficial spreading melanoma	29	27	0	2
Total	65	61	2	2
Benign Nevi				
Blue nevus	3	0	0	3
Compound nevus	5	0	1	4
Deep penetrating nevus	6	1	1	4
Intradermal nevus	4	0	0	4
Junctional nevus	3	1	1	1
Dysplastic nevus with mild atypia	19	2	0	17
Dysplastic nevus with moderate atypia	16	1	3	12
Spitz nevus	6	2	1	3
Total	62	7	7	48

Abbreviation: NOS, not otherwise specified.

Table 3 Results of gene expression testing by clinical follow-up

Test result	Histopathology diagnosis	Clinical outcomes	
		Metastases	No adverse events
Melanoma			
Malignant	61	14	47
Indeterminate	2	0	2
Benign	2	0	2
Total	65	14	51
Benign Nevi			
Malignant	7	0	7
Indeterminate	7	0	7
Benign	48	0	48
Total	62	0	62

Validation studies that evaluate these ancillary tests in cases with documented clinical outcomes have been limited in scope [9,18-20], and most use histopathologic diagnosis as the reference standard. The 23-gene signature was initially validated using a cohort of 437 lesions for which 2 dermatopathologists independently rendered the same diagnosis [11]. Similar results were observed in a second validation study using 736 lesions for which 3 dermatopathologists had independently arrived at the same diagnosis [12]. Use of consensus histopathologic diagnosis as the reference standard is a practical approach when outcome data are unavailable, but it is limited by the subjectivity inherent in classification by histopathology. Validation using lesions for which there are well-documented clinical outcomes is a more rigorous method. Here, the gene expression signature results were compared to actual clinical outcomes in 127 melanocytic neoplasms from in the initial validation study.

2. Materials and methods

2.1. Sample cohort

This study included a subset of samples that were used in the initial validation study, which was performed with an institutional review board–approved waiver for consent (Quorum Review, Seattle, WA) [11]. Archival formalin-fixed paraffin-embedded tissue sections were selected by expert dermatopathologists to represent a broad range of histopathologic subtypes. Submitting dermatopathologists provided a diagnosis of nevus or melanoma, as well the histopathologic subtype. Subtypes were assigned by an independent dermatopathologist (L. E. C.) for cases that were submitted without a subtype or that required adjudication. Re-excision specimens were excluded. For this study, the subset of cases submitted from 1 institution (Cleveland Clinic, Cleveland, OH) was evaluated (n = 127).

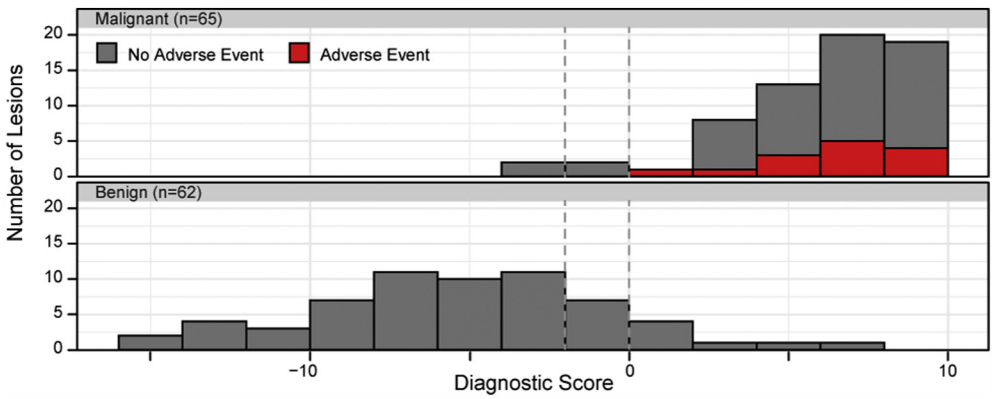

Fig. 1 Distribution of gene expression signature scores. Scores ≥0 are likely malignant, scores between −0.1 and −2.0 are indeterminate, and scores ≤−2.1 are likely benign. All metastatic cases (red) received a malignant test result.

Clinical follow-up was obtained by medical record review and was shared in a randomized, deidentified fashion with oversight by the institutional review board of the Cleveland Clinic. Clinical and/or histopathologic evidence of local recurrence, sentinel lymph node metastasis, and distant metastases was recorded.

2.2. Gene expression signature testing

Gene expression signature testing has been previously described [11,21]. In brief, an anatomic pathologist identified representative sections of each lesion from a hematoxylin and eosin (H&E)–stained slide. The corresponding area was macrodissected from unstained tissue, and RNA was extracted. The differential expression of 14 tumor marker genes and 9 housekeeper genes was measured using quantitative reverse-transcription polymerase chain reaction. The tumor marker genes include genes associated with cell differentiation (*PRAME*), tumor microenvironment cell signaling (*S100A7*, *S100A8*, *S100A9*, *S100A12*, *P13*), and tumor immune response signaling (*CCL5*, *CD38*, *CXCL10*, *CXCL9*, *IRF1*, *LCP2*, *PTPRC*, *SELL*). A weighting algorithm was used to normalize the average expression of the tumor marker genes to the housekeeper genes and generate a single numeric score. Scores from −16.7 to −2.1 were reported as "likely benign," scores from −2.0 to −0.1 were reported as "indeterminate," and scores from 0.0 to +11.1 were reported as "likely malignant."

2.3. Performance of the gene expression signature assay

As part of the protocol for the initial validation study, each lesion was independently reviewed by a second dermatopathologist who was blinded to the gene expression test results and the diagnosis of the original (submitting) dermatopathologist [11]. Only lesions that received concordant diagnoses of "benign" or "malignant" by both dermatopathologists were included in that study. In the current analysis, the results of gene expression testing were correlated with the presence or absence of adverse events (*adverse events* were defined as local recurrence, sentinel lymph node metastasis, and/or distant metastases).

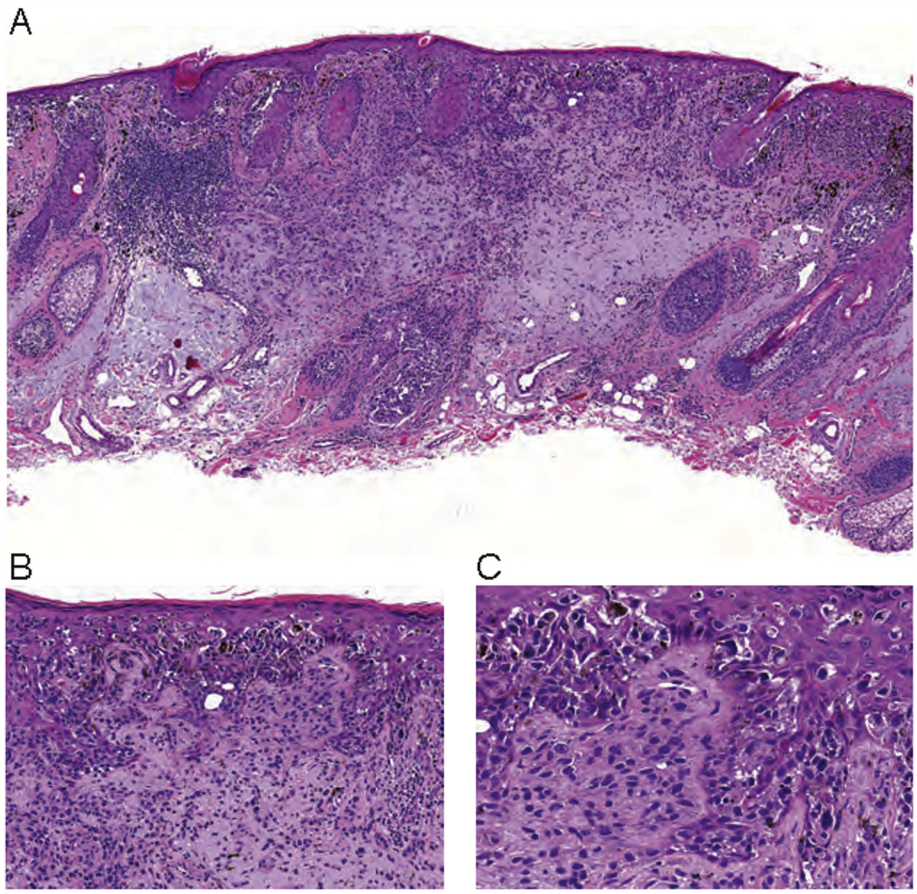

Fig. 2 H&E-stained slides at (A) 5×, (B) 20×, and (C) 40×. This lesion from the face of an 83-year-old man received a diagnosis of lentigo maligna melanoma by histopathology. The gene expression score was +4.2 ("likely malignant"). Brain metastases developed, and the patient died 37 months after the original biopsy.

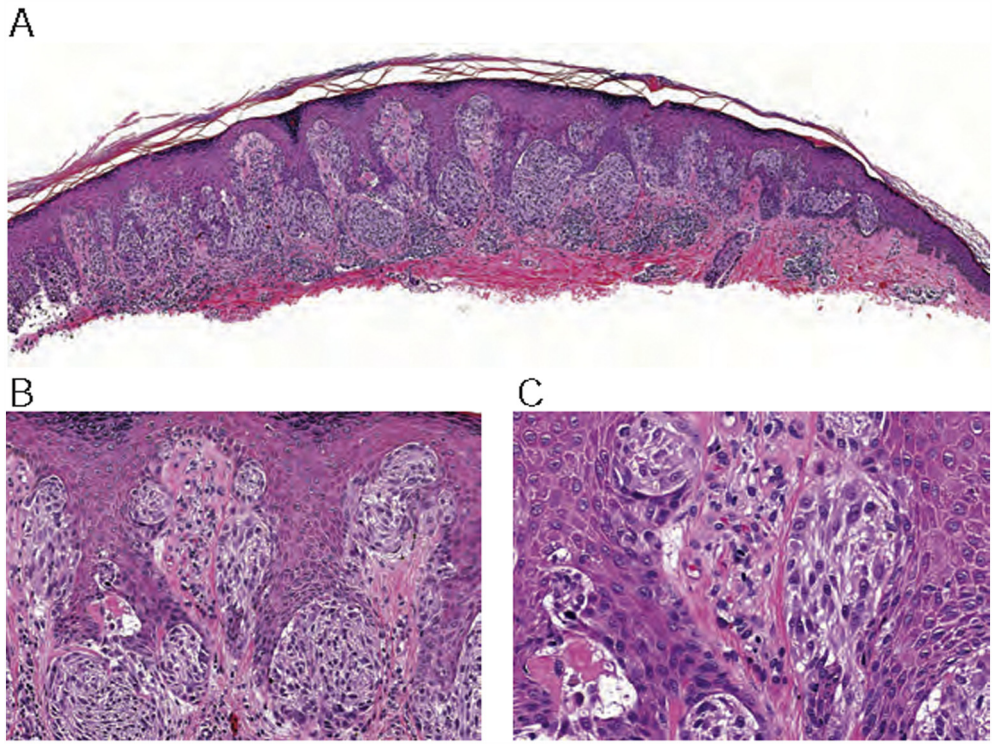

Fig. 3 H&E-stained slides at (A) 5×, (B) 20×, and (C) 40×. This lesion from the lower extremity of a 27-year-old woman received a diagnosis of Spitz nevus. The gene expression score was −3.8 ("likely benign"). There was no evidence of disease at 38 months.

The performance of the test was quantitatively evaluated in cases with definitive clinical outcomes. Because this cohort was not selected based on clinical outcomes and the expected time frame for biologic progression of melanoma exceeds the duration of follow-up here, the absence of an event cannot be used to unequivocally define a lesion as benign. Therefore, the sensitivity (ability to identify true positives) of the gene expression signature was evaluated only in the subset of cases that went on to metastasize.

Discordant cases for which the gene expression assay disagreed with the histopathologic diagnosis (apparent false positive and false negatives) were qualitatively reviewed. H&E-stained sections of all discordant cases, all cases with an indeterminate test result, and a subset of concordant cases underwent additional review by 3 experienced dermatopathologists (J. S. K., S. D. B., and L. E. C.). Dermatopathologists were blinded to the test result, the initial diagnoses, and whether the case was discordant or concordant with histopathologic diagnosis.

3. Results

3.1. Lesions diagnosed as melanoma by histopathology

Overall, 65 (51.2%) lesions were diagnosed as malignant melanoma by histopathology. The clinical characteristics are provided in Table 1. Fourteen (21.5%) of those 65 cases went on to develop metastases, including 6 lesions with positive sentinel lymph node biopsies, 5 lesions with distant metastases, and 3 lesions with both positive sentinel lymph node biopsies and distant metastases. The median follow-up time for cases diagnosed as malignant with and without metastasis was 14 and 48 months, respectively. The average age for patients with melanomas that metastasized was 58 years compared to 66 years for patients with histopathologic diagnoses of melanoma but no evidence of metastasis during follow-up. The median Breslow depth for the cases diagnosed as malignant with and without evidence of metastasis was 1.76 and 1.25 mm, respectively. Ulceration was present in 50% of the melanomas that metastasized and in 18% of non-metastasizing lesions that were diagnosed as melanoma histopathologically.

Among cases diagnosed as malignant, 10 subtypes were included (Table 2), with superficial spreading melanoma (n = 29), nodular melanoma (n = 18), and lentigo maligna melanoma (n = 8) being the most common. Less common subtypes included acral melanoma not otherwise specified, acral lentiginous melanoma, and desmoplastic melanoma.

A malignant gene expression test result was produced by 61 of 65 (93.8%) lesions diagnosed histopathologically as melanoma (Table 3). All 14 cases that went on to metastasize produced a malignant result (Fig. 1), and a representative case is shown in Fig. 2. The resulting sensitivity of the gene

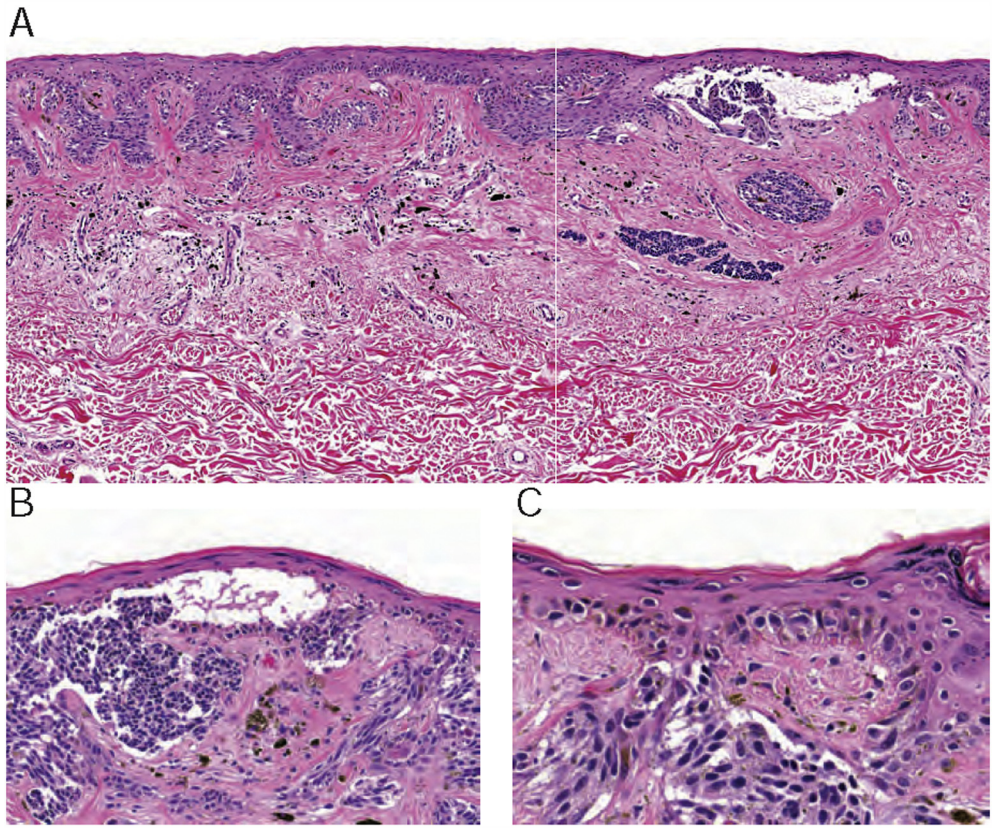

Fig. 4 H&E-stained slides at (A) 5×, (B) 20×, and (C) 40×. This lesion from the trunk of a 64-year-old woman was initially diagnosed as a dysplastic nevus with moderate atypia. The gene expression score was +3.9 ("likely malignant"). There was no evidence of disease at 51 months, and therefore, this lesion was classified as a false positive. At a subsequent review by 3 dermatopathologists (who were blinded to the score and the original diagnosis), the favored diagnosis was melanoma.

signature among cases that metastasized was, therefore, 100%. No adverse events (local recurrence or metastasis) were detected in the remaining 47 cases that were diagnosed histopathologically as melanoma and produced a malignant result by gene expression testing (Table 3). Gene expression testing produced an indeterminate result for 2 lesions that were diagnosed as melanoma by histopathology. Both of these were classified as desmoplastic melanomas; neither developed local recurrence or metastasis. Benign gene expression test results were produced by 2 lesions diagnosed histopathologically as superficial spreading melanomas (Table 2). There was no recurrence/metastasis in these apparent false negatives after 41 and 95 months of follow-up.

3.2. Lesions diagnosed as benign by histopathology

In this cohort, 62 (48.8%) lesions were diagnosed as benign by histopathology (Table 1). No evidence of local recurrence or metastasis was observed for any of these cases. The median follow-up time was 30 months. The average age of patients with a benign lesion was 42 years. Eight nevus subtypes were included, with the most common being nevi with "mild" (n = 19) or "moderate" (n = 16) dysplasia (Table 2).

Among lesions that received a benign diagnosis by histopathology, 48 of 62 (77.4%) produced a benign score (Fig. 1, Table 3). This included several subtypes that are sometimes difficult to diagnose by histopathology alone, such as Spitz nevi (Fig. 3). Indeterminate scores were produced by 7 lesions classified as nevi by histopathology. These included 3 dysplastic nevi with moderate dysplasia and 1 Spitz nevus (Table 2). Malignant scores were produced for 7 of 62 (11.3%) benign lesions, including 1 lesion classified as a deep penetrating nevus, 2 classified as Spitz nevi, and 2 classified as dysplastic nevi (Table 2). The follow-up time for these apparent false positives ranged from 26 to 69 months.

An informal post hoc blinded histopathologic review was performed that included concordant cases and cases in which the gene signature result was discordant with the initial histopathologic diagnosis. The lesion shown in Fig. 4 is an apparent false positive that was considered suspicious for melanoma upon review by the participating dermatopathologists. Fig. 5 shows a case where additional review was concordant with the initial diagnosis of benign nevus.

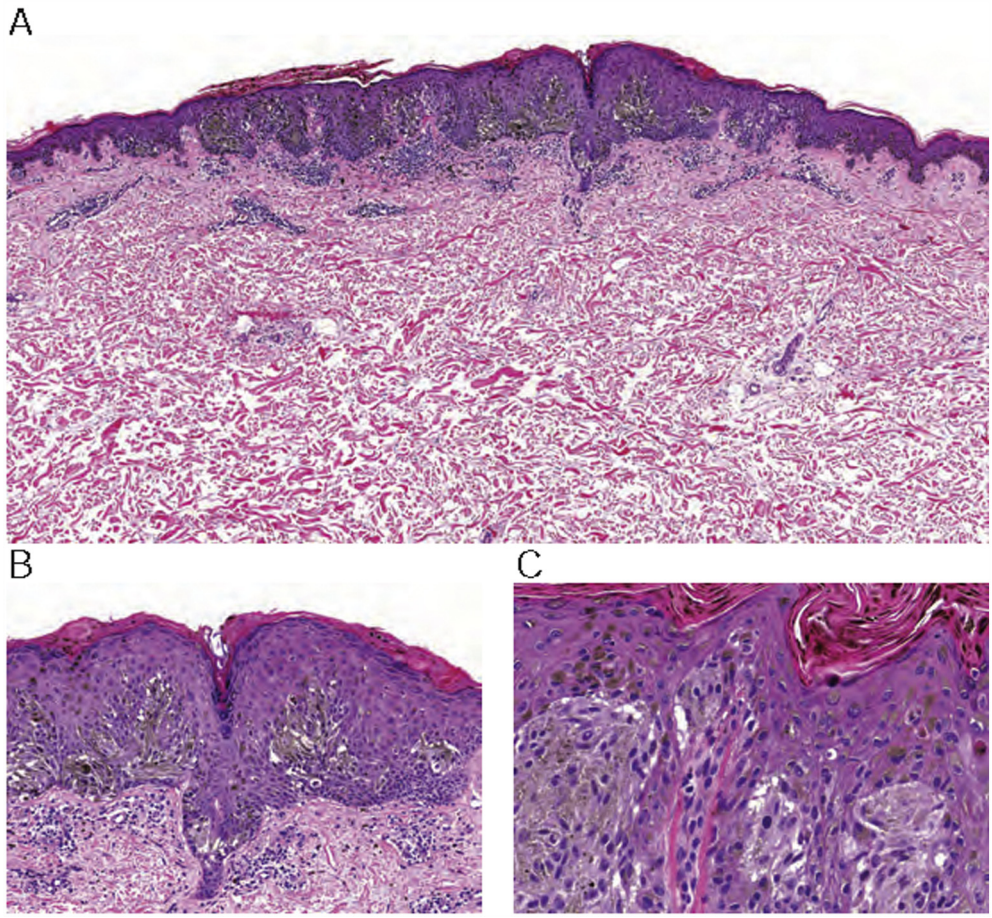

Fig. 5 H&E-stained slides at (A) 5×, (B) 20×, and (C) 40×. This lesion from the lower extremity of a 43-year-old woman received a diagnosis of Spitz nevus. The gene expression score was +4.1 ("likely malignant"). There was no evidence of disease at 28 months. Based on the benign clinical outcome, this was therefore considered a false-positive result.

4. Discussion

Although histopathologic interpretation is accurate for the diagnosis of most melanocytic neoplasms, adjunctive molecular tools have been developed to aid in the classification of lesions that are ambiguous by histopathology. A 23-gene expression signature was previously shown to differentiate melanoma from nevi with sensitivity and specificity of greater than 90% by comparison to histopathologic diagnosis [11]. Diagnosis by histopathologic interpretation can serve as a valid reference standard, especially when there is concordance among multiple observers, but the inherent subjectivity remains a limitation, and even diagnostic concordance does not guarantee accuracy. Therefore, this study sought to evaluate the diagnostic accuracy of the gene expression signature by comparison to actual clinical outcomes for a subset of lesions with concordant histopathologic diagnoses included in the first validation study.

The cohort included 65 neoplasms diagnosed as primary cutaneous melanoma by histopathology. Fourteen of the 65 ultimately developed metastases, all of which were correctly identified as malignant by the gene expression signature. False-negative scores were produced for 2 of the 65 lesions. Both were diagnosed as superficial spreading melanoma by histopathology, and features that might account for the discordant scores were not readily apparent by microscopy. However, it is reassuring that follow-up time for one of the lesions was over 7 years and no adverse events occurred during this time. Two lesions produced indeterminate scores, both of which were classified as desmoplastic melanoma histopathologically. This result is in keeping with previous studies showing that the sensitivity of ancillary tests, including single nucleotide polymorphism arrays [22] and FISH [23], is likely lower for desmoplastic melanoma than for other melanoma subtypes.

The 62 lesions diagnosed as nevi by histopathology had a median follow-up of 30 months. None recurred or metastasized during that time. The majority (77.4%) produced benign gene expression scores and represent true negatives. Specificity for this 62-lesion subset is 88.7% (95% confidence interval: 78.1%-95.3%) and does not differ significantly from the previously reported 91% (95% confidence interval: 87.0%-95.0%) specificity for the full cohort [11]. Seven lesions produced positive scores, and because none had evidence of recurrence or

metastasis during the follow-up period, these were classified as false positives. However, in an informal blinded histopathologic review conducted by 3 experienced dermatopathologists at a multiheaded microscope, 2 of the 7 apparent false-positive cases were considered suspicious for melanoma by all reviewers. Although follow-up did not reveal any adverse events, it is possible that these lesions represent melanomas that were cured by the initial excision or that metastasis did not develop during the follow-up period. Specific histopathologic or clinical characteristics that might account for the unexpected scores were not identified at the histopathologic review.

In the evaluation of melanocytic lesions, a foremost objective of dermatopathologists is accurate classification of lesions with biologic potential for recurrence or distant spread. This is primarily because simple excision is curative for many lesions that show borderline histopathologic features. Overall, the sensitivity of the gene expression signature among cases that metastasized was 100%. It is noteworthy that this cohort was not selected based on outcomes and the malignant subset of cases was based on histopathologic diagnosis. Given that metastases may develop many years after the initial diagnosis of melanoma or may not develop at all, the absence of an adverse event among cases diagnosed as malignant by histopathology here (median follow-up 48 months) does not mean that the histopathologic diagnosis of melanoma is incorrect. Nevertheless, the intent of the current study is to evaluate the gene expression signature among biologically proven melanomas, in particular, in a cohort of unselected cases. Similarly, the absence of an event among cases diagnosed as benign by histopathology (median follow-up 30 months) cannot definitively confirm that a lesion is benign. However, the performance of the gene signature here among unselected lesions that metastasized is consistent with a recent study of a large cohort of melanocytic lesions selected based on clinical outcomes. In that study, the gene expression signature showed a sensitivity of 93.8% in lesions diagnosed as malignant based on distant metastases [24]. The previous study also demonstrated a specificity of 96.2% in lesions diagnosed as benign based on long-term, disease-free follow-up [24].

Several limitations of this study warrant mention. First, none of the lesions in this cohort were classified as severely dysplastic nevi, nor were any of the 6 Spitz nevi considered "atypical" by the participating dermatopathologists. Performance of the assay in these and other subtypes that may have "borderline" biological potential therefore warrants further study. Second, although all of the cases that metastasized were apparently correctly diagnosed histopathologically by the submitting dermatopathologists, a rigorous analysis of their potential for ambiguity (such as a blinded review by 5 or more dermatopathologists to calculate potential discordance) was not undertaken. As such, the potential for these lesions to generate diagnostic discordance or be considered ambiguous by histopathologic interpretation is unknown.

To validate a diagnostic test, its performance must be evaluated relative to the current diagnostic standard for the disease state. Although previous studies have evaluated the gene expression against the current "gold" standard for melanoma diagnosis, histopathology, there are known limitations with diagnostic concordance among dermatopathologists. Another approach is to validate the gene signature against long-term clinical outcomes. Although distant metastasis of a melanocytic lesion definitively identifies a lesion as malignant, the fact that some melanomas metastasize many years after initial diagnosis and many others do not metastasize at all makes it challenging to definitively classify lesions without an adverse event. As such, a combined approach evaluating the gene expression assay against histopathology and outcomes can provide a more complete understanding of the test performance. To this end, the data presented here show a strong association between the long-term clinical outcomes and the gene expression signature test results in this cohort. Importantly, no false-negative test results were produced by any of the melanomas that ultimately developed metastases.

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