Associations Between Pigmentation and the Clinicopathologic and Immune Landscape of Melanoma

Helana Ghali, BS, Ayah N. Al-Bzour, MD,† Aleena Boby, BA,* Shaliz Aflatooni, BS,* Danielle K. DePalo, MD,‡ Michelle M. Dugan, MD,§ Emily Coughlin, MPH,¶ Rahul Mhaskar, PhD, MPH,¶ Kenneth Tsai, MD, PhD,§‖ Jonathan S. Zager, MD,§** Vernon K. Sondak, MD,§ Sanjay Premi, PhD,†† Walter J. Storkus, PhD,‡‡ Jane L. Messina, MD,§‖ and Lilit Karapetyan, MD, MS, FACP§§§*

Abstract: Pigmentation varies widely in cutaneous melanoma, but its clinicopathologic and immunologic implications are incompletely defined. We retrospectively reviewed 627 patients with stage II–III melanoma treated at H. Lee Moffitt Cancer Center (2010–2019) and correlated pathologist-graded tumor pigmentation (0–3) with tumor features and sentinel lymph node biopsy results. We then evaluated transcript levels of pigmentation markers (TYR, DCT, MITF) for associations with overall survival (OS), tumor microenvironment (TME) composition, and immune checkpoint inhibitor (ICI) response using TCGA-SKCM bulk RNA-seq (n = 443) and public cohorts (GSE91061, GSE115978, GSE120575). Pigmented tumors (74.2%) showed lower Breslow depth ($P = 0.001$), more tumor-infiltrating lymphocytes ($P < 0.001$), greater angiolymphatic invasion ($P = 0.03$), enrichment for superficial spreading subtype ($P < 0.001$), and higher sentinel lymph node biopsy positivity ($P < 0.05$) versus nonpigmented tumors. In TCGA, high TYR, DCT, and MITF expression independently correlated with worse OS. xCell analysis showed that low expression groups were enriched for multiple effector T- and B-cell populations, whereas high expression associated with immunosuppressive cell types (e.g., M2 macrophages, Tregs). In an ICI-treated cohort (GSE91061), baseline MITF was higher in nonresponders ($P = 0.023$). Single-cell analyses confirmed pigmentation gene expression in malignant cells and demonstrated elevated MITF within myeloid subsets of nonresponders. Overall, both histologic pigmentation and pigmentation-gene signatures associate with tumor characteristics, immune contexture, and clinical outcomes, suggesting potential utility for risk stratification and treatment response prediction.

Key Words: melanoma, pigmentation, tyrosinase, tumor microenvironment, immune checkpoint inhibitors

(*Am J Dermatopathol* 2026;48:439–448)

From the *University of South Florida Morsani College of Medicine, Tampa, FL; †Faculty of Medicine, Jordan University of Science and Technology, Irbid, Jordan; ‡Department of General Surgery, University of Massachusetts Chan Medical School, Boston, MA; §Department of Cutaneous Oncology, H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL; ¶Department of Medical Education, University of South Florida Morsani College of Medicine, Tampa, FL; ‖Department of Pathology, H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL; **Department of Oncologic Sciences, University of South Florida Morsani College of Medicine, Tampa, FL; ††Department of Tumor Microenvironment and Metastasis (TMEM), H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL; ‡‡Departments of Immunology and Dermatology, University of Pittsburgh School of Medicine, Pittsburgh, PA; and §§Immuno-oncology program, H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL.

Dr. J. S. Zager has received payments from Delcath Systems—Medical Advisory Board. He has consulting agreements with Philogen, Merit Medical, Castle Biosciences, and Merck. His department receives research funding from Philogen, SWOG, Delcath Systems, and Provectus. Dr. K. Tsai has consulting agreements with Verrica Pharmeceuticals, Senhwa Bsciences, NFlection Therapeutics, and has received research funding from Incyte Corporation and Merk. Dr. V. K. Sondak has consulting agreements with Bristol Myers Squibb, Genesis Drug Discovery & Development, Iovance, Merck, Mural Oncology, Novartis, and Sun Pharmaceuticals; research funding from Neogene Therapeutics, Skyline, and Turnstone. Dr. L. Karapetyan has received research support from Iovance Biotherapeutics to her institution, Valar labs to her institution; and traveling support from Immatics.

H. Ghali, A. Al-Bzour, A. Boby, S. Aflatooni, Dr. D. K. DePalo, Dr. M. M. Dugan, E. Coughlin, Dr. R. Mhaskar, Dr. J. L. Messina, Dr. W. Storkus and Dr. S. Premi have no conflicts of interest or financial ties to disclose.

This study was conducted under IRB approval [MCC #22519/ IRB #Pro00071072.].

Correspondence: Helana Ghali, MD, Morsani College of Medicine, University of South Florida, 12901 Bruce B Downs Blvd. Dr, Tampa, FL 33612 (e-mail: ghali2@usf.edu).

INTRODUCTION

Melanin is produced by melanocytes in the basal epidermis within cytosolic melanosomes, which are transferred to keratinocytes in skin for host protection against the deleterious effects of ultraviolet (UV) radiation.[1] UV radiation serves as the principal stimulus for melanogenesis and the subsequent production of melanosomes.[2] Melanin exists in several forms and mediates diverse biologic functions, notably contributing to skin and hair pigmentation and providing photoprotection for the skin and eyes. It also exhibits well-established antioxidative properties, functioning as a free radical scavenger to protect normal melanocytes from the cytopathic effects of UV radiation and oxidative stress.[3]

However, melanogenesis can also produce reactive intermediates with genotoxic and mutagenic potential, which may contribute to the initiation and progression of melanoma.[4–7] Investigations into the role of tumor pigmentation in determining clinical outcome have yielded mixed results. Melanin-rich neoplastic melanocytes exhibit reduced sensitivity to chemotherapy, radiotherapy, and photodynamic therapy, and patients presenting with amelanotic melanomas

exhibit longer disease-free and overall survival when compared with patients with melanotic melanomas because of increased sensitivity to intervention-induced tumor cell death.[8] Consequently, interventional inhibition of melanogenesis has been proposed as a potential cotherapeutic strategy for cutaneous melanomas. Conversely, other reports suggest that amelanotic melanomas are more frequently diagnosed at advanced clinical stages in association with poorer survival outcomes, thus highlighting the complex and potentially context-dependent role of pigmentation in melanoma progression and outcomes prognosis.[9]

At some specialized institutions, the degree of tumor pigmentation is routinely documented as part of the standard pathologic evaluation of primary cutaneous melanomas to facilitate further research in this arena. Despite existing biologic insights, the relationship between tumor pigmentation in cutaneous melanoma and key clinicopathologic and histopathologic features—such as Breslow depth, histologic subtypes, and tumor-infiltrating lymphocytes (TILs)—remains unclear, limiting our understanding of their potential disease significance. The aim of this study was to evaluate these associations to clarify the relationship between tumor pigmentation on other primary cutaneous melanoma characteristics in a large series of tumors treated at a single institution. A secondary aim was to evaluate the association between tumor pigmentation markers and clinical outcomes by analyzing expression of tyrosinase (*TYR*), Dopachrome Tautomerase (*DCT*), and Melanocyte Inducing Transcription Factor (*MITF*) as transcriptomic markers of tumor pigmentation in the TCGA-SKCM cohort, and to explore their correlation with patient survival outcomes, response to immune checkpoint inhibitors (ICIs), and tumor microenvironment (TME) features to provide molecular support for implementing tumor pigmentation as an actionable prognostic biomarker in cutaneous melanoma.

MATERIALS AND METHODS

This study was approved by the Moffitt Cancer Center Scientific Review Committee (protocol # MCC #22519/IRB #Pro00071072). A single-institution retrospective chart review including all adult patients with stage II-III primary invasive melanoma treated with excision and sentinel node biopsy at Moffitt Cancer Center from 2010 to 2019 was conducted. Patients were excluded if they did not undergo a sentinel lymph node biopsy, had mucosal melanoma, had multiple primary melanomas, or had clinical stage III or IV disease at the time of diagnosis. All primary melanomas included in this study were reviewed, with their diagnoses confirmed at Moffitt Cancer Center, where pigmentation grading was also performed.

Data were collected through retrospective chart review. All patient data were deidentified. Patient records from outside institutions were concurrently reviewed when available. A diagnosis of melanoma was confirmed based on clinical documentation and dermatopathology reports. Pigmentation was categorized on a scale from 0 to 3: 0 indicates no visible pigment; 1 denotes faint, sparse intracytoplasmic melanin visible at 20× magnification or higher; 2 represents abundant melanin visible at 10× magnification or lower; and 3 indicates heavy intracytoplasmic melanin that obscures nuclear details (Fig. 1).

The presence and degree of primary tumor pigmentation was compared across variables using Pearson's χ^2 and Kruskal–Wallis tests for categorical variables and Mann–Whitney U test for continuous variables. Statistical tests were 2-sided, and significance was defined as P-value of <0.05. All statistical analyses were conducted using R version 4.2.

Pigmentation was compared with key clinicopathologic variables, including Breslow depth, ulceration, angiolymphatic invasion, histologic subtype, the presence and degree of tumor-infiltrating lymphocytes (TILs), and sentinel lymph node biopsy (SLNB) positivity.

Transcriptional Analysis of Pigmentation Marker Genes Using the TCGA-SKCM Cohort

To assess the clinical and prognostic relevance of pigmentation in melanoma, we investigated associations between transcript levels of pigmentation marker genes including Tyrosinase (*TYR*), Dopachrome Tautomerase (*DCT*), and Melanocyte Inducing Transcription Factor (*MITF*) deduced from bulk RNA-sequencing data from The Cancer Genome Atlas (TCGA-SKCM) database of 443 melanoma patients with corresponding patient-matched clinical and overall survival (OS) data. Gene expression levels were log2-transformed and summarized using median and interquartile range (IQR). The median gene expression values were used to stratify samples into high versus low expression groups. Kaplan–Meier plots with median OS (95% CI) and log-rank tests were used to assess prognostic significance. To assess how pigmentation marker gene expression levels

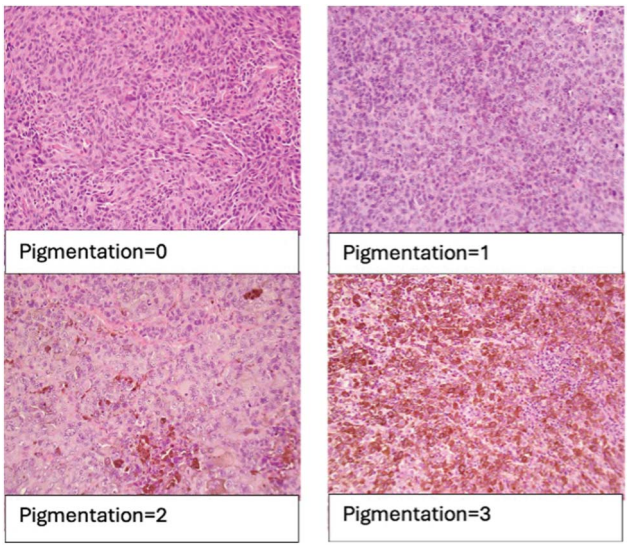

FIGURE 1. Melanoma tumor pigmentation scale, with 0 indicating no visible pigment; 1 indicating faint, sparse intracytoplasmic melanin visible at 20× magnification or higher; 2 representing abundant melanin visible at 10× magnification or lower; and 3 representing heavy intracytoplasmic melanin that obscures nuclear details.

correlate with each other, we used Pearson correlations analysis for comparisons between marker genes. To compare the association between gene expression levels and clinical data (sample types [primary vs. metastatic] and sample sources [skin vs. lymph node]), we used the Wilcoxon rank-sum test.

To evaluate associations between immune cell infiltration and TME biomarkers versus pigmentation markers, we applied the xCell algorithm to the TCGA-SKCM cohort to estimate the relative abundance of 62 immune and stromal cell types. Differences in immune cell abundance between high and low expression groups for each marker were compared using the Wilcoxon test.

To determine whether pigmentation markers are associated with resistance to immunotherapy, we next analyzed gene expression levels in a melanoma cohort treated with ICIs by using the data from GSE91061 cohort, which includes bulk RNA-seq data from 56 ICI-treated patients with melanoma (13 responders and 43 nonresponders). Expression of pigmentation markers was compared between responders and nonresponders using the Wilcoxon test.

Finally, to explore the cellular context of pigmentation markers at single-cell (scRNA) resolution, we analyzed 2 publicly accessible scRNA data sets from the Gene Expression Omnibus. The GSE115978 data set included a total of 7186 cells from 31 ICI-treated melanoma patients with available meta-data on cell type and source (lymph node vs. malignant tumor). The GSE120575 data set consists of 16,291 cells from 48 ICI-treated melanoma patients with available data on response status. Raw data were processed using the Seurat v4.0 package in R. Differential expression analysis between cell types and response groups was performed using Seurat's FindMarkers function, with significance based on $\log_2$ fold change ($\log_2$FC) and adjusted *P*-values (Benjamini–Hochberg method).

RESULTS

A total of 627 retrospective institutional patients were identified (Table 1), with 64.4% being men (n = 404), with a median age of 65 years, and 77.5% of patients having stage II disease (n = 486). Tumor pigmentation was graded as 0 in 25.8% (n = 162), 1 in 50.7% (n = 318), 2 in 21.5% (n = 135), and 3 in 1.9% (n = 12) of patients evaluated. Mean Breslow depth of all tumors was 3.46 mm (SD 2.28), 52.9% (n = 332) of tumors were ulcerated, 7.7% (n = 48) of cases exhibited angiolymphatic invasion, and 36% (n = 225) of lesions were of the nodular histologic subtype.

Compared with tumors without pigmentation, the presence of any degree of pigmentation was associated with a lower Breslow depth (mean 3.3 ± 2.8 vs. 4.1 ± 2.8 mm, $P = 0.001$), the presence of TILs (91.6% vs. 70%, $P < 0.001$), evidence of angiolymphatic invasion (9.1% vs. 3.8%, $P = 0.029$), and superficial spreading subtype (46.3% vs. 25.5%, $P < 0.001$; Fig. 2). Patients with pigmented tumors were more likely to have a positive sentinel lymph node biopsy than those with nonpigmented tumors (25.2% vs. 14.8%, $P = 0.007$). When stratified by degree of pigmentation, patients with tumors exhibiting a lower degree of pigmentation (degree of 1) were significantly older than patients with tumors graded as highly pigmented (degree of 3), with mean ages of 64.8 ± 14.0 years versus 55.7 ± 13.9 years, respectively ($P < 0.001$).

Associations Between Pigmentation Marker Expression and Patient Survival, Tumor Microenvironment Composition, and Response to ICI

Using independent separate melanoma cohorts from publicly available data sets, we analyzed the transcriptomic association of pigmentation marker genes with prognosis, tumor microenvironment, and ICI response. To explore the prognostic value of pigmentation marker gene expression deduced from bulk-RNA sequencing data, we used the TCGA-SKCM cohort that included a total of 443 SKCM samples with available survival data. The median (IQR) age at initial diagnosis was 54.0 (47.0–70.0) years, with 62% being men (n = 274). The majority (83%) of sample types were metastatic (n = 367), with 221 (50%) taken from lymph nodes, with 222 (50%) representing skin samples. Median *TYR*, *DCT*, and *MITF* expression levels were 13.7 (12.4–14.5), 11.7 (8.5–13.5), and 12.4 (11.6–13.0), respectively.

Median OS was 79.0 (95% CI: 64.4–103.0), with a significantly worse OS in the high-*TYR* group (Median OS [95% CI]: 65.9 [50.8–89.1] vs. 103.3 [79.0–152.8], $P = 0.015$; Figure 3A). Similarly, the high-*DCT* group had a significantly worse OS compared with the low-*DCT* group (Median OS [95% CI]: 65.9 [54.4–96.2] vs. 103.1 [66.7–167.9], $P = 0.013$; Figure 3B). The high-*MITF* group also had a significantly worse OS compared with the low-*MITF* group (Median OS [95% CI]: 66.7 [50.8–93.0] vs. 103.3 [66.5–168.0], $P = 0.012$; Figure 3C). Therefore, our results suggest that pigmentation-associated markers (*TYR, MITF,* and *DCT*) show a consistent association with overall survival, with higher expression levels correlating with worse survival compared with their respective low-expression groups.

We next investigated correlative expression of the pigmentation markers with each other. There was a significant strong direct correlation between *TYR* and *DCT* (correlation coefficient = 0.67, $P < 0.001$; Fig. 3D), a significant positive correlation between *TYR* and *MITF* (correlation coefficient = 0.77, $P < 0.001$; Fig. 3D), and between *DCT* and *MITF* (correlation coefficient = 0.72, $P < 0.001$; Fig. 3D).

In addition, we investigated the expression of pigmentation marker genes between sample types (primary vs. metastatic) and sample sources (lymph node vs. skin). *TYR* expression was significantly higher in primary samples (median (IQR): 14.1 (13.3–15.1) versus 13.6 (12.0–14.4), $P = 0.004$; Figure 3E). However, there was no significant difference in *TYR* expression between skin and lymph node samples ($P = 0.095$; Fig. 3F). In addition, there was no significant difference in *DCT* and *MITF* expression levels between primary versus metastatic samples or skin versus lymph node samples ($P > 0.05$; Fig. 3E-F).

We used xCell algorithm to assess the tumor microenvironment between pigmentation markers in the TCGA-SKCM cohort. In the low-*TYR* group, there was a significantly higher abundance of $CD4^+$ and $CD4^+$ memory T cells, class-switched

TABLE 1. Baseline Patient and Tumor Characteristics

	Presence of Pigmentation		Degree of Pigmentation		
	No N = 162 (%)	Yes N = 465 (%)	1 N = 318 (%)	2 N = 135 (%)	3 N = 12 (%)
Age (mean ± SD)	64.8 ± 14.3	63.0 ± 14.9	64.8 ± 14.0	59.4 ± 16.3	55.7 ± 13.9
Sex					
Male	105 (64.8)	299 (64.3)	203 (63.8)	87 (64.4)	9 (75.0)
Female	57 (35.2)	166 (35.7)	115 (36.2)	48 (35.6)	3 (25.0)
Histology					
Nodular	76 (53.9)	150 (34.2)	97 (32.4)	47 (36.4)	6 (60.0)
Superficial spreading	36 (25.5)	203 (46.3)	132 (44.1)	67 (51.9)	4 (40.0)
Desmoplastic	16 (11.3)	37 (8.4)	35 (11.7)	2 (1.6)	0 (0)
Other	13 (9.2)	48 (11.0)	35 (11.7)	13 (10.1)	0 (0)
Breslow depth (mm)	4.1 ± 2.8	3.3 ± 2.8	3.3 ± 2.4	3.3 ± 2.1	3.4 ± 1.7
Mitoses	6.1 ± 6.5	6.7 ± 7.4	6.7 ± 7.5	6.8 ± 7.5	4.3 ± 4.7
TILs					
None	48 (30.0)	39 (8.4)	25 (7.9)	12 (8.9)	2 (16.7)
Nonbrisk	105 (65.6)	344 (74.1)	238 (75.1)	98 (72.6)	8 (66.7)
Brisk	7 (4.4)	81 (17.5)	54 (17.0)	25 (18.5)	2 (16.7)
Ulceration	95 (58.6)	237 (51.0)	167 (52.5)	65 (48.1)	5 (41.7)
Regression	9 (5.6)	47 (10.1)	28 (8.8)	18 (13.3)	1 (8.3)
Perineural invasion	4 (3.6)	14 (3.7)	13 (5.1)	1 (0.9)	0 (0)
Angiolymphatic invasion	6 (3.8)	42 (9.1)	23 (7.3)	18 (13.3)	1 (8.3)
Microsatellites	1 (0.6)	6 (1.4)	3 (1.0)	3 (2.4)	0 (0)
SLNB					
Negative	138 (85.2)	348 (74.8)	245 (77.0)	96 (71.1)	7 (58.3)
Positive	24 (14.8)	117 (25.2)	73 (23.0)	39 (28.9)	5 (41.7)
BRAF mutation	22 (57.9)	59 (62.8)	31 (57.4)	25 (67.6)	3 (100)

TIL, tumor-infiltrating lymphocytes; SLNB, sentinel lymph node biopsy.

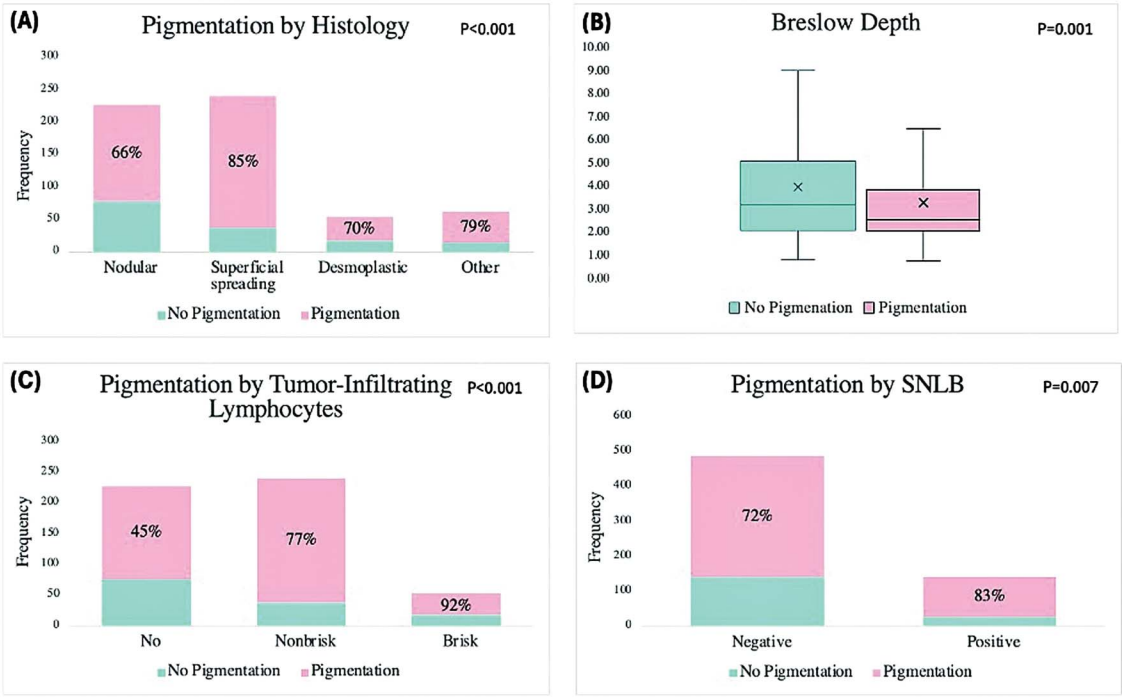

FIGURE 2. Associations between presence of pigmentation in primary melanoma lesion and (A) histologic subtype, (B) Breslow depth, (C) TILs, and (D) SLNB. SLNB, sentinel lymph node biopsy; TIL, tumor-infiltrating lymphocytes.

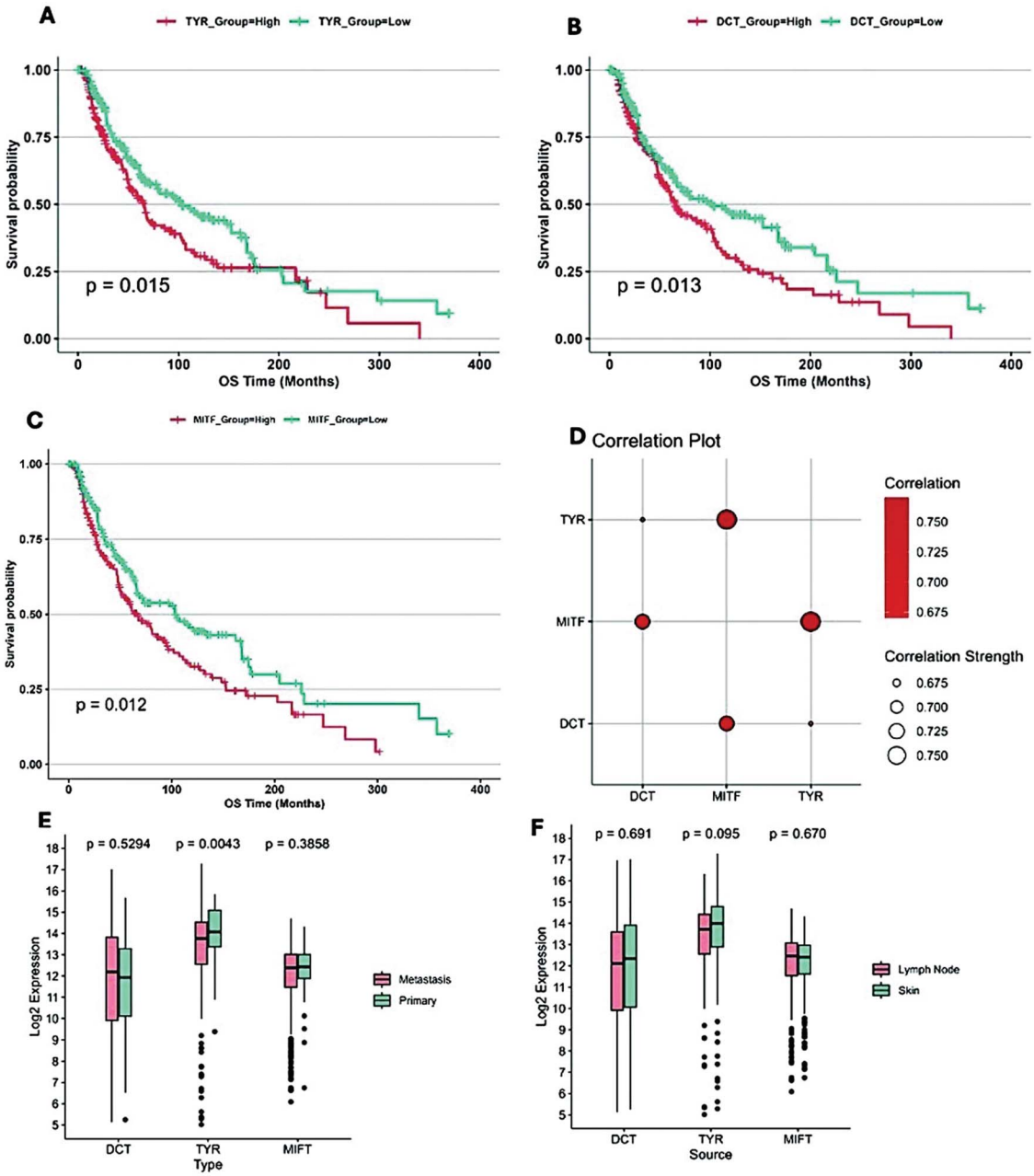

FIGURE 3. Analysis of transcriptomic pigmentation markers (*TYR*, *DCT*, and *MITF*) in public data sets. A, KM plot for OS between *TYR* group in the TCGA-SKCM data set, (B) KM plot for OS between *DCT* group in the TCGA-SKCM data set, (C) KM plot for OS between *MITF* group in the TCGA-SKCM data set, (D) Pearson correlation between pigmentation markers in the TCGA-SKCM cohort, (E) boxplot showing difference in gene expression between sample types in the TCGA-SKCM cohort, and (F) boxplot showing differences in gene expression between sample source. DCT, Dopachrome Tautomerase; KM, Kaplan–Meier; MITF, Melanocyte Inducing Transcription Factor; OS, overall survival; SKCM, cutaneous melanoma; TCGA, The Cancer Genome Atlas; TYR, tyrosinase.

memory B cells (CSM B cells), endothelial cells, fibroblasts, natural killer cells (NKC), and T-helper 2 (Th2) cells (Fig. 4A). Whereas the high-*TYR* group had a significantly higher abundance of protumor (M2) macrophages, and T-helper 1 (Th1) cells. In the low-*DCT* group, there was a significantly higher abundance of $CD4^+$ effector-memory T-cells (Tem), $CD8^+$ Tem, CSM B-cells, endothelial cells, macrophages, memory B-cells, and plasma cells. Whereas the high-*DCT* group had a significantly higher abundance of basophils (Fig. 4B). In the low-*MITF* group, there was a significantly higher abundance of basophils, naive $CD4^+$ T cells, $CD4^+$ central memory (Tcm), $CD4^+$ Tem, $CD8^+$ T cells, $CD8^+$ Tcm, $CD8^+$ Tem, CSM

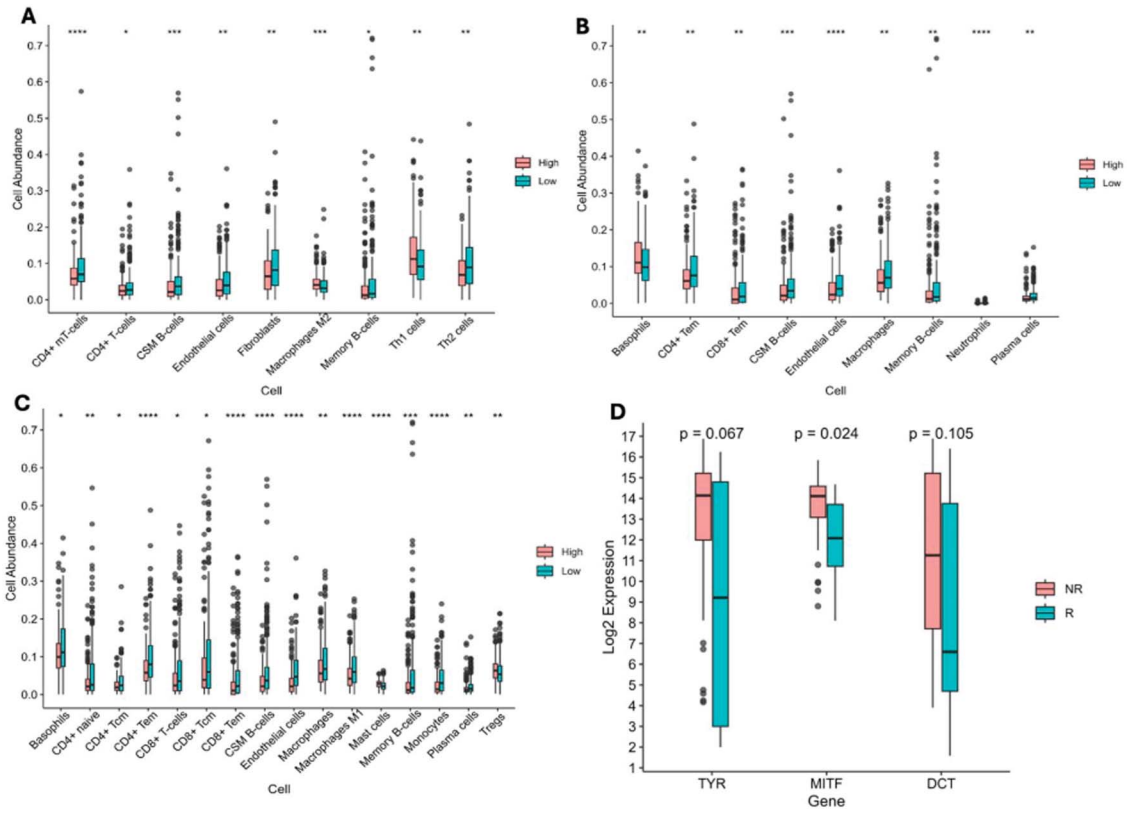

FIGURE 4. Immune landscape association with pigmentation markers (*TYR*, *DCT*, and *MITF*) in public data sets. A, TME analysis using xCell algorithm in the TCGA-SKCM cohort between *TYR* groups, (B) TME analysis using xCell algorithm in the TCGA-SKCM cohort between *DCT* groups, (C) TME analysis using xCell algorithm in the TCGA-SKCM cohort between *MITF* groups, and (D) comparison of pigmentation markers expression between ICI-responders versus nonresponders in the (GSE91061) cohort. **P*-value <0.05, ***P*-value <0.01, ****P*-value <0.001, *****P*-value <0.0001. DCT, Dopachrome Tautomerase; ICI, immune checkpoint inhibitors; MITF, Melanocyte Inducing Transcription Factor; SKCM, cutaneous melanoma; TCGA, The Cancer Genome Atlas; TME, tumor microenvironment; *TYR*, tyrosinase.

B cells, endothelial cells, fibroblasts, macrophages, memory B cells, plasma cells. Whereas the high-*MITF* group had a significantly higher abundance of regulatory T cells (Tregs), and mast cells (Fig. 4C).

To investigate the role of pigmentation markers between responders and nonresponders in ICI-treated patients with melanoma, we used the GSE91061 data set that included a total of 56 on-ICI patients with melanoma (43 nonresponders, and 13 responders). There was a significantly higher expression of *MITF* in baseline tumors from nonresponders (median (IQR): 14.11 (13.09–14.59) versus 12.08 (10.73–13.71), $P = 0.023$; Fig. 4D). In contrast, *TYR* expression (median (IQR): 14.1 (12.0–15.2) versus 9.2 (3.0, 14.8), $P = 0.068$) and *DCT* expression (median (IQR): 11.3 (7.7–15.2) versus 6.6 (4.7–13.8), $P = 0.11$) did not reach statistical significance between nonresponders and responders, although both showed a trend toward higher expression in nonresponders. Taken together, these findings suggest that increased pigmentation marker expression, particularly *MITF*, may be associated with reduced ICI responsiveness.

We next explored the single-cell landscape using the SKCM melanoma scRNAseq data set (GSE115978) that included a total of 7186 cells for 31 ICI-treated patients and (GSE120575) data set that included a total of 16,291 cells of 48 patients treated with ICIs. In the GSE115978 data set, *TYR* was detectable in malignant cells ($Log_2FC = 2.09$) and significantly upregulated in lymph node clusters ($Log_2FC = 0.59$; Fig. 5A). Similarly, *DCT* was significantly detectable in malignant cells ($Log_2FC = 2.68$), and downregulated in lymph node cluster ($Log_2FC = -0.59$, Fig. 5B). MITF was also significantly upregulated in malignant cells (Log2FC = 1.52), while no significant difference was noted between lymph node and malignant tumor clusters (Fig. 5C). In the GSE120575 data set, there was no significant differential expression of *TYR* and *DCT* between cell clusters (Figs. 5D, E), while *MITF* was significantly upregulated in macrophages ($log_2FC = 0.37$). In addition, *MITF* was significantly upregulated in ICI nonresponders within monocyte and macrophage clusters ($Log_2FC = 0.25$; Fig. 5F).

DISCUSSION

Cutaneous melanoma arises from pigment-producing melanocytes, and studies suggest that the degree of

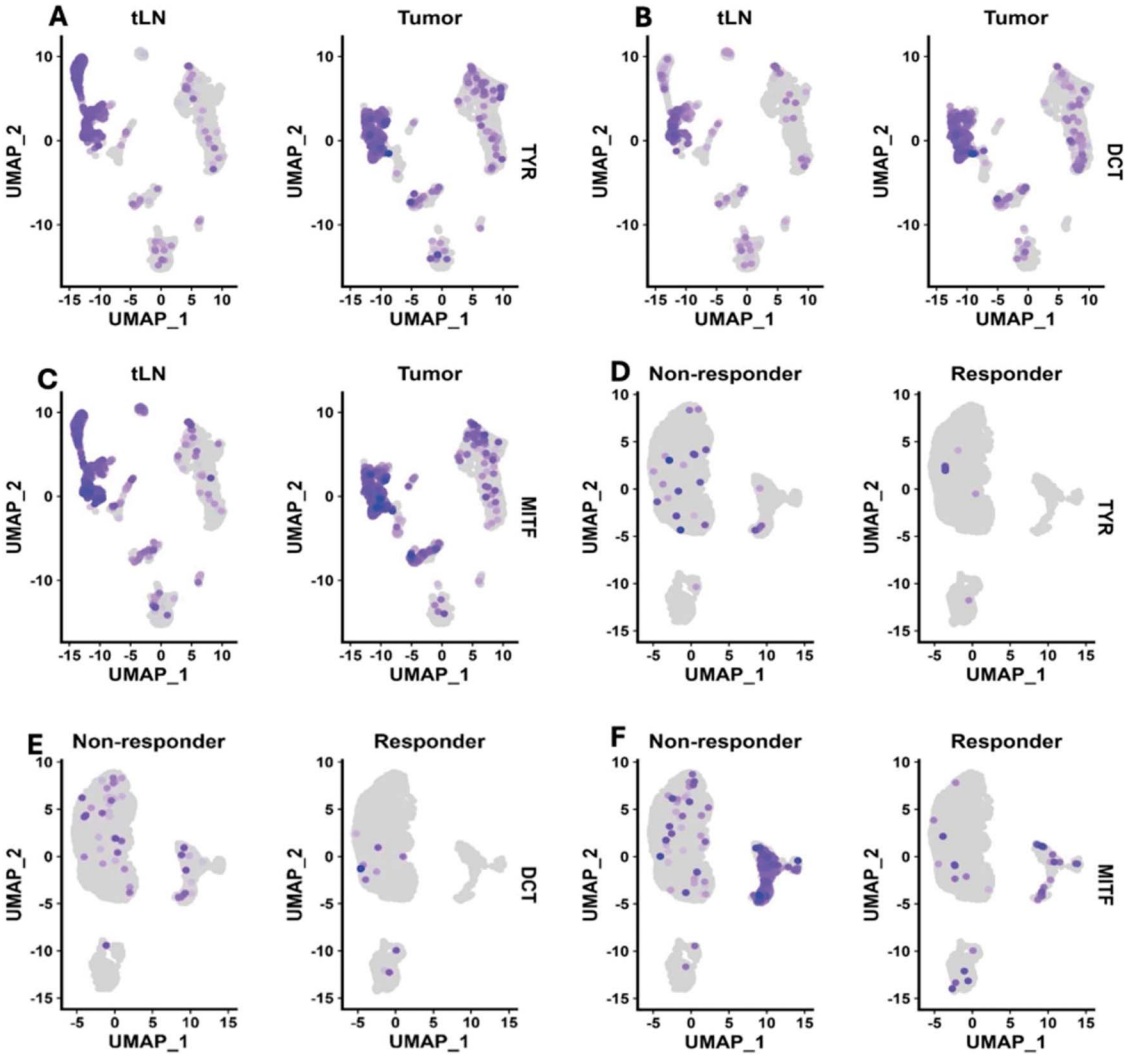

FIGURE 5. Single-cell landscape of pigmentation markers (*TYR*, *DCT*, and *MITF*) using public data sets. A–C, feature plots of *TYR*, *DCT*, and *MITF* between cell source (tumor lymph node [tLN] vs. malignant tumor cells) in the GSE115978 data set, respectively. D–F, Feature plots of *TYR*, *DCT*, and *MITF* between ICI responders versus nonresponders in the GSE120575 data set, respectively. DCT, Dopachrome Tautomerase; ICI: immune checkpoint inhibitors; MITF, Melanocyte Inducing Transcription Factor; TYR, tyrosinase.

pigmentation may play a role in tumor pathogenicity.[9,10] Tumors with higher melanin production have also been associated with treatment resistance, possibly related to differences in the immunogenicity profile.[4,5] Unfortunately, the contextual literature is limited, and the impact of tumor pigmentation on patient prognosis outcomes remains poorly understood. This study examined a large number of patients with melanoma treated at a single cancer center involving expert dermatopathology review that routinely reports degree of tumor pigmentation among the primary melanoma characteristics. In addition, using independent publicly available data sets, we evaluated the prognostic and immunologic significance of pigmentation marker gene products namely *TYR*, *DCT*, and *MITF* using transcriptomic analyses.

Institutional Cohort

Given our institutional cohort represents primary melanomas evaluated histopathologically, whereas the TCGA-SKCM data set consists predominantly of metastatic tumors analyzed using transcriptomic methods, the biologic context and prognostic implications of pigmentation differ between these data sets. For this reason, we present and interpret the findings from each cohort separately. Our study found that when compared with nonpigmented tumors, pigmented melanomas were associated with a superficial spreading histology, a lower Breslow depth, presence of TILs (most commonly brisk), angiolymphatic invasion, and sentinel lymph node-positive micrometastatic disease. These findings confirm and extend notable differences in primary melanoma tumor characteristics based on pigmentation status.[11,12]

Some reports have described amelanotic melanomas as being associated with worse survival outcomes.[10,13–15] Our findings support the hypothesis that nonpigmented lesions exhibit more aggressive tumor pathologic features, including greater Breslow depth and absence of TIL. Conversely, nonpigmented lesions were more often sentinel node negative and lacked angiolymphatic invasion. This interplay of conflicting prognostic markers likely accounts for the lack of conclusive evidence on the role of pigmentation in the literature. For example, Wee et al[14] initially found an association between amelanotic melanoma and worse melanoma-specific survival, however, this was not statistically significant after accounting for Breslow depth.

TCGA-SKCM Cohort

In addition, our analysis of the TCGA-SKCM cohort showed that higher expression levels of *TYR*, *DCT*, and *MITF*, which are markers of melanocytic differentiation, melanin synthesis, and pigmentation, are associated with significantly worse survival outcomes. This can potentially be explained by the finding that tumors with lower *TYR, DCT,* and *MITF* expression showed a higher immune activity, as evidenced by greater infiltration by memory $CD4^+$ T cells, $CD8^+$ T cells, and B cells, suggesting that reduced melanocytic differentiation and pigmentation may coincide with a more immune-active and proinflammatory TME, potentially explaining improved outcomes despite histologically aggressive features in nonpigmented tumors. These findings can also be explained by the higher abundance of M2 macrophages (proangiogenic inflammatory cells), which have been associated with worse survival outcomes in melanoma with higher pigmentation levels.[16] In the study by Jiang et al,[17] MITF was upregulated in secreted phosphoprotein 1 (*SPP+*) lipid-associated macrophages (LAMs), which significantly contribute to the downregulation of the mitogen-activated protein kinase (MAPK) pathway and further enhance the inflammatory response. Therefore, these markers may serve as independent prognostic indicators and identify high-risk patients who may benefit from closer monitoring or personalized management. Importantly, these results challenge the assumption that more pigmented tumors are inherently less aggressive, underscoring the need to integrate molecular and histopathologic data in prognostic assessment.[18]

Our primary cohort, however, found no significant differences in recurrence-free survival or OS between pigmented and nonpigmented tumors, despite differences in pathologic features such as histologic subtype, Breslow depth, and the presence of TIL. These results suggest that the presence of TILs alone may not fully reflect functional antitumor immunity. Although our transcriptomic analysis of the TCGA showed that lower expression of pigmentation markers was associated with higher inferred immune activity, our institutional analysis showed that clinically pigmented lesions may contain higher number of TILs. These findings may not be mutually exclusive. Bulk RNA-based immune estimates reflect functional immune activation, whereas histologic TIL assessments quantify total immune cell presence, which may include exhausted or ineffective TILs. Indeed, highly pigmented tumors may harbor increased number of TILs that do not translate into productive antitumor immunity, potentially reflecting immune evasion or exhaustion. Thus, although certain TIL phenotypes seemed prognostically favorable, they displayed features of exhaustion—evidenced by marker expression and reduced cytokine production.[19,20] This distinction highlights the importance of differentiating between TIL quantity and functional immune engagement, and further studies integrating histology with functional single-cell or spatial transcriptomics are warranted.

Furthermore, our results suggest that higher *MITF* expression is significantly associated with lower ICI response, while *TYR* and *DCT* demonstrated a similar but nonsignificant trend. Although not all pigmentation markers reached statistical significance, the overall pattern implies that higher pigmentation markers may be linked to reduced ICI effectiveness and a less immune-infiltrated TME warranting further validation in larger ICI-treated cohorts.

It has been shown that melanin plays a crucial role in the progression of melanoma, with hyperpigmentation being associated with resistance to various therapies, including ICIs. A preclinical study by Huang et al[21] further supports the immunosuppressive role of tyrosinase in melanoma. Using a CRISPR/Cas9-generated tyrosinase-knockout B16 melanoma model, tumor loss of TYR expression resulted in increased T-cell infiltration and activation within the TME, indicating that tyrosinase expression suppresses antitumor immunity. Andreucci et al showed that miR-214-overexpressing (miR-214^+) melanoma cells exhibit hyperpigmentation linked to reduced sensitivity to chemotherapy, targeted therapy, and radiotherapy in vitro. In addition, inhibition of miR-214 signaling or melanogenesis restored treatment responsiveness. Furthermore, elevated plasma levels of miR-214 were observed in ICI-resistant patients with melanoma highlighting its potential role in mediating resistance.[22]

Across transcriptomic data sets, higher expression of pigmentation-associated genes was linked with less favorable immune features and reduced responsiveness to immune checkpoint inhibitors; however, because our institutional cohort did not assess survival differences by pigmentation status, these associations should be interpreted cautiously and validated across homogeneous data sets. Because tumor pigmentation is not routinely reported in metastatic disease, future studies should include histologic grading of metastatic samples to enable further investigations. Hopefully pigment-related indices may eventually be incorporated into shared decision-making discussions to help patients understand the prognostic implications of their tumor pathology. A personalized treatment approach involving multidisciplinary collaboration informed by such discussions remains essential to the effective management of melanoma in the clinical setting.

When considered together, the institutional and transcriptomic findings should not be viewed as contradictory, but rather reflective of distinct disease settings and analytic modalities. Histologic pigmentation in primary tumors was associated with more favorable clinicopathologic features such as lower Breslow depth and superficial spreading subtype, whereas high pigmentation-marker expression in predominantly metastatic TCGA samples corresponded with reduced immune activation and poorer overall survival. These

differences likely reflect stage-related biology, cohort composition, and the inherent differences between morphologic and transcriptomic measures of pigmentation.

It is also important to consider heavily pigmented melanocytic neoplasms such as the entity previously termed "animal-type melanoma" (now more commonly classified under the spectrum of melanocytoma).[23–25] These tumors characteristically demonstrate dense melanin pigmentation, low-to-intermediate mutational burden, and a generally indolent but sometimes unpredictable clinical course.[26–28] Their distinct biology illustrates that heavy pigmentation does not uniformly correlate with aggressive behavior, and highlights the heterogeneity of pigment-related pathways across melanocytic tumors.[23,24,26,29–31] Including such entities in the broader context of pigmentation biology reinforces the need for careful integration of morphologic features, molecular data, and clinical behavior when interpreting the prognostic significance of tumor pigmentation.

Limitations

Limitations of this study include its retrospective nature. Statistical analyses were somewhat limited by lower sample size in the stage III patient group. Furthermore, although our institution has specialty dermatopathology resources, this may not be available in all clinical settings, limiting the operational utility of our results. In addition, although comparison of pathologic prognostic features was performed on primary melanomas, the outcomes comparisons were predominantly performed on metastatic samples. Although every effort was made to retrieve updated patient information, some patients were ultimately lost to follow-up. Despite these limitations, this study contributes valuable insights regarding the impact of melanoma pigmentation on clinicopathologic outcomes.

CONCLUSIONS

Tumor pigmentation in melanoma reflects underlying biologic differences in the TME that are associated with both clinicopathologic features and survival outcomes. Our findings suggest that pigmentation and related pigment-associated gene expression signatures may be prognostic for worse prognosis in terms of overall survival and ICI responsiveness in melanoma cohorts, and that tumors with lower levels of pigmentation exhibit a proinflammatory environment that may serve as prognostic biomarkers that offer insights into tumor immune interactions with clinical implications. Our findings suggest that pigmentation status and pigmentation-related gene expression may have prognostic relevance; however, additional studies in larger and more homogeneous cohorts are needed before these markers can be incorporated into formal risk-stratification models.

REFERENCES

1. D'Alba L, Shawkey MD. Melanosomes: biogenesis, properties, and evolution of an ancient organelle. *Physiol Rev*. 2019;99:1–19.
2. Del Bino S, Duval C, Bernerd F. Clinical and biological characterization of skin pigmentation diversity and its consequences on UV impact. *Int J Mol Sci*. 2018;19:2668.
3. Brenner M, Hearing VJ. The protective role of melanin against UV damage in human skin. *Photochem Photobiol*. 2008;84:539–549.
4. Slominski A, Tobin DJ, Shibahara S, et al. Melanin pigmentation in mammalian skin and its hormonal regulation. *Physiol Rev*. 2004;84:1155–1228.
5. Slominski RM, Zmijewski MA, Slominski AT. The role of melanin pigment in melanoma. *Exp Dermatol*. 2015;24:258–259.
6. Meyskens FL, Farmer PJ, Yang S, et al. New perspectives on melanoma pathogenesis and chemoprevention. *Recent Results Cancer Res*. 2007;174:191–195.
7. Kollias N, Sayre RM, Zeise L, et al. Photoprotection by melanin. *J Photochem Photobiol B*. 1991;9:135–160.
8. Brożyna AA, Jóźwicki W, Carlson JA, et al. Melanogenesis affects overall and disease-free survival in patients with stage III and IV melanoma. *Hum Pathol*. 2013;44:2071–2074.
9. Brożyna AA, Jóźwicki W, Roszkowski K, et al. Melanin content in melanoma metastases affects the outcome of radiotherapy. *Oncotarget*. 2016;7:17844–17853.
10. Guo W, Yin G, Liu H, et al. Matched analysis of the prognosis of amelanotic and pigmented melanoma in head and neck. *Acta Otolaryngol*. 2020;140:785–788.
11. Dedeilia A, Lwin T, Li S, et al. Factors affecting recurrence and survival for patients with high-risk stage II melanoma. *Ann Surg Oncol*. 2024;31:2713–2726.
12. Falkenius J, Keskitalo J, Kanter L, et al. A biomarker panel predicts recurrence-free survival in ulcerated primary cutaneous melanoma. *Acta Oncol (Madr)*. 2022;61:14–21.
13. Thomas NE, Kricker A, Waxweiler WT, et al. Comparison of clinicopathologic features and survival of histopathologically amelanotic and pigmented melanomas: a population-based study. *JAMA Dermatol*. 2014;150:1306–1314.
14. Wee E, Wolfe R, Mclean C, et al. Clinically amelanotic or hypomelanotic melanoma: anatomic distribution, risk factors, and survival. *J Am Acad Dermatol*. 2018;79:645–651.e4.
15. Gong HZ, Zheng HY, Li J. Amelanotic melanoma. *Melanoma Res*. 2019;29:221–230.
16. Jha J, Singh MK, Singh L, et al. Deciphering the intricate relationship between macrophages, pigmentation, and prognosis in uveal melanoma. *Lab Invest J Tech Methods Pathol*. 2024;104:102167.
17. Jiang Y, Yu W, Hu T, et al. Unveiling macrophage diversity in myocardial ischemia-reperfusion injury: identification of a distinct lipid-associated macrophage subset. *Front Immunol*. 2024;15:1335333.
18. Aebischer V, Abu-Ghazaleh A, Metzler G, et al. Histopathologic abundance of pigmentation correlates with disease-specific survival in malignant melanoma but is not independent of current AJCC pT stage. *Pigment Cell Melanoma Res*. 2023;36:512–521.
19. Noorwali H. Tumor-infiltrating lymphocytes in melanoma: diagnostic and prognostic implications from biopsy to circulation. *The J Liquid Biopsy*. 2025;9:100308.
20. deLeeuw RJ, Kroeger DR, Kost SE, et al. CD25 identifies a subset of CD4+FoxP3− TIL that are exhausted yet prognostically favorable in human ovarian cancer. *Cancer Immunol Res*. 2015;3:245–253.
21. Huang R, Wang Y, Teng H, et al. Tyrosinase in melanoma inhibits antitumor activity of PD-1 deficient T cells. *BMC Biol*. 2025;23:135.
22. Andreucci E, Ruzzolini J, Bianchini F, et al. miR-214-Enriched extracellular vesicles released by acid-adapted melanoma cells promote inflammatory macrophage-dependent tumor trans-endothelial migration. *Cancers (Basel)*. 2022;14:5090.
23. Ludgate MW, Fullen DR, Lee J, et al. Animal-type melanoma: a clinical and histopathological study of 22 cases from a single institution. *Br J Dermatol*. 2010;162:129–136.
24. Vyas R, Keller JJ, Honda K, et al. A systematic review and meta-analysis of animal-type melanoma. *J Am Acad Dermatol*. 2015;73:1031–1039.
25. Pampena R, Lai M, Lombardi M, et al. Clinical and dermoscopic features associated with difficult-to-recognize variants of cutaneous melanoma: a systematic review. *JAMA Dermatol*. 2020;156:430–439.
26. Antony FC, Sanclemente G, Shaikh H, et al. Pigment synthesizing melanoma (so-called animal type melanoma): a clinicopathological study of 14 cases of a poorly known distinctive variant of melanoma. *Histopathology*. 2006;48:754–762.

27. Isales MC, Mohan LS, Quan VL, et al. Distinct genomic patterns in pigmented epithelioid Melanocytoma: a molecular and histologic analysis of 16 cases. *Am J Surg Pathol*. 2019;43:480–488.
28. Cohen JN, Yeh I, Mully TW, et al. Genomic and clinicopathologic characteristics of PRKAR1A-inactivated melanomas: toward genetic distinctions of animal-type melanoma/pigment synthesizing melanoma. *Am J Surg Pathol*. 2020;44:805–816.
29. Zembowicz A, Carney JA, Mihm MC. Pigmented epithelioid melanocytoma: a low-grade melanocytic tumor with metastatic potential indistinguishable from animal-type melanoma and epithelioid blue nevus. *Am J Surg Pathol*. 2004;28:31–40.
30. Bax MJ, Brown MD, Rothberg PG, et al. Pigmented epithelioid melanocytoma (animal-type melanoma): an institutional experience. *J Am Acad Dermatol*. 2017;77:328–332.
31. Crowson AN, Magro CM, Mihm MC. Malignant melanoma with prominent pigment synthesis: "animal type" melanoma--a clinical and histological study of six cases with a consideration of other melanocytic neoplasms with prominent pigment synthesis. *Hum Pathol*. 1999;30:543–550.