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STAT6 Expression Correlates with Melanin Pigmentation in Melanomas

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Abstract

Background: The melanin pigment may play a significant role in influencing melanoma invasion, metastatic potential, and tumor response to therapy. Melanogenesis, the biological process responsible for melanin production, can be suppressed through activation of the Janus kinase-signal transducer and activator of transcription 6 (JAK-STAT6) signaling pathway, which results in a reduction of melanin levels. Given this mechanism, we aimed to investigate the possible association between STAT6 expression and the degree of melanin pigmentation in patients diagnosed with primary cutaneous melanomas. **Methods:** Analysis of mRNA expression data was conducted in a cohort of 329 melanoma tumors from The Cancer Genome Atlas (TCGA) dataset, and the relationship between STAT6 transcriptional levels and pigmentation levels was assessed. STAT6 protein content was evaluated by immunohistochemistry (IHC) in 91 tumor samples, and the results were correlated with pigmentation levels observed histologically. **Results:** Pigmentation scores varied significantly among groups with different STAT6 expression levels for both mRNA and protein expression (mRNA, pigment scores 1 and 2, both $p < 0.05$ versus pigment score 0; pigment score 3, $p < 0.0001$ versus pigment score 0; protein, pigmented versus non-pigmented tumors, $p = 0.039$). **Conclusion:** These findings suggest a potential association between gene and protein expression of STAT6 and melanin pigmentation levels in primary melanomas, suggesting a possible role for STAT6 in pigmentation regulation.

1 Introduction

Sun exposure plays a fundamental role in melanogenesis and the development of melanoma. Human behavior regarding sun exposure is strongly influenced by the climate, culture, and overall environment where people live. Worldwide, there are significant variations in these factors, as well as diverse skin phototypes [1]. Melanoma pigmentation, a clinically relevant factor for diagnosis, presents distinct characteristics according to age group. The literature reports that melanomas in adults are generally more pigmented, while amelanotic forms are more frequently observed in pediatric patients, proportionally to the total number of melanomas in each population [2].

Melanin can influence the invasive and metastatic potential of melanomas and may also play a role in the therapeutic response [3,4]. Melanogenesis is determined by complex interactions among various biological functions [5]. Melanin synthesis requires the effective coordination of metabolic pathways across multiple intracellular compartments. While pigment production provides a communal protection from UV damage, it also imposes anabolic and redox demands that must be carefully managed by melanocytes [6].

Melanoma is recognized to exhibit phenotypic plasticity and capacity to transdifferentiate along vascular and neural lineages [7]. Depending on the stage of disease, melanoma cells exhibit phenotypes resembling those of neural crest cells. These phenotypes are regulated by specific signaling pathways and transcription factors, including the microphthalmia-associated transcription factor (MITF), which plays a key role in controlling pigmentation [8-10]. In normal melanocytes, MITF can be inhibited by the Janus kinase-signal transducer and activator of transcription 6 (JAK-STAT6) pathway, leading to a reduction in melanogenesis [11,12].

The JAK-STAT pathway regulates the signaling of almost all immune processes, including those related in vitiligo and tumor cell recognition [13,14]. The STAT6 transcription factor plays a central role in the development of different tumors, regulating the tumor microenvironment [15,16], and tumor initiation and progression, especially in lymphomas and solitary fibrous tumors [13]. In addition, this transcription factor seems to be involved in the survival of gliomas and its epigenetic restoration could offer a potential alternative therapy for these tumors [17].

Interleukin-4 (IL-4) inhibits the melanogenesis of normal human melanocytes through a mechanism dependent on JAK2-STAT6 signaling [11], and STAT6 activation is found in IL-4/Luc/CNS-1 transgenic mice harboring B16F10 melanoma tumors and treated with phthalic anhydride [18]. In experimental melanoma, metastatic capacity is influenced by STAT6 [19]. Expression of STAT genes, including STAT6, has been proposed as a potential prognostic biomarker in cutaneous melanoma [20], and the prognostic significance of JAK-STAT signaling pathway genes in melanoma has been investigated [12]. Inherited abnormal genotypes in genes encoding components of the JAK/STAT pathway are associated with higher risk of cutaneous melanoma [21]. The aim of the present study was to evaluate the association of STAT6 expression with melanin pigmentation in patients with primary melanoma.

2 Materials and methods

2.1 Gene expression analysis

An analysis of STAT6 transcription levels was conducted using data from the melanoma set of The Cancer Genome Atlas (TCGA) portal (https://tcga-data.nci.nih.gov/tcga). The expression of STAT6 mRNA was evaluated in relation to melanin pigmentation, based on data from 329 samples available in the dataset [22].

2.2 Immunohistochemistry

Immunohistochemical analysis of STAT6 protein expression was performed in primary melanomas thicker than 1.0 mm, with a total of 91 samples from patients of both sexes. Tissue samples were obtained from patients from the municipal public health network who were diagnosed with primary melanoma between 2011 and 2015. The samples were derived from whole tumors embedded in paraffin blocks stored at the local institutional tissue bank. Histological slides of all samples were stained with hematoxylin-eosin, and the presence of melanin pigmentation was assessed by two observers. Immunohistochemical expression of STAT6 was determined in the nucleus and cytoplasm. Two observers, who were blinded to the patient's clinical data, analyzed the slides. A rabbit anti-STAT6 monoclonal antibody (clone EP325, CellMarqueTM Tissue Diagnosis, Darmstadt, Germany), diluted 1: 100 was used for immunhistochemistry. Immunohistochemical staining was performed in an automated system (Autostainer Link 48, Dako, Glostrup, Denmark). Samples of solitary fibrous tumor were used as positive controls. Normal skin keratinocytes present in non-tumoral adjacent tissue within the same samples served as negative controls. The reaction was developed by incubating the slides with the EnVision FLEX HRP Magenta Substrate Chromogen System (Dako) for 5 minutes. The H-score was calculated by multiplying the staining intensity (0: no staining; 1: weak staining; 2: moderate staining; 3: strong staining) by the percentage of stained tumor cells in 10% intervals, resulting in values of 0 to 300. Samples with an H-score above the median were classified as positive [23].

2.3 Statistical methods

The expression of mRNA was normalized on the R2 Genomics Analysis and Visualization Platform (http://r2.amc.nl) and is presented in boxplots as $\log_2$-transformed signal intensity. Comparisons of mRNA levels among groups were performed with Kruskal-Wallis test followed by Tukey tests; p values of less than 0.05 indicated significant differences between groups.

3 Results

3.1 Gene expression of STAT6 increases with melanoma pigmentation

Clinicopathological characteristics of the patients included in the immunohistochemical analysis are summarized in Table 1. Analysis of data for the correlation between transcriptional levels of STAT6 and melanin pigmentation score in a dataset of 329 melanomas revealed that STAT6 mRNA levels increased significantly with higher pigmentation scores compared to cases with no pigmentation (pigment scores 1 and 2, both $p < 0.05$ versus pigment score 0; pigment score 3, $p < 0.0001$ versus pigment score 0; Figure 1). There were no significant differences in STAT6 expression among different disease stages (data not shown).

3.2. STAT6 protein content increases with melanoma pigmentation

In the primary melanomas selected for STAT6 immunohistochemical analysis, immunohistochemical expression of STAT6 (H-score > 5) was detected in 86 (94.5%) samples, and the median STAT6 expression score was 85. Among the 91 tumors, 70 (76.9%) showed pigmentation, and STAT6 expression above the median was observed in 38 (54.3%) of these samples. Conversely, 6 (28.6%) of the 21 (23.1% of total) non-pigmented samples exhibited expression above the median. The difference observed between these groups was statistically significant ($p = 0.039$). Figure 2 shows representative samples of immunohistochemical expression of STAT6 in normal skin, solitary fibrous tumor and melanomas with H $\leqslant$ 85 and > 85.

Table 1 Clinicopathological features of patients in immunohistochemical analysis.

Indicators		Primary melanomas, STAT6, n (%)
Gender	Male	45 (49.5)
	Female	46 (50.5)
Mean age (SD), years		(91 ± 16.3)
Tumor location Primary melanoma	Trunk	39 (42.9)
	Head and neck	17 (18.7)
	Upper limbs	17 (18.7)
	Lower limbs	18 (19.8)
TNM stage	IIa	27 (29.7)
	IIb	2 (2.2)
	IIIa	6 (6.6)
	IIIb	13 (14.3)
	IVa	12 (13.2)
	IVb	31 (34.1)
Histological type	Superficial dissemination	38 (41.8)
	Nodular	45 (49.5)
	LM/LMM	0 (0)
	Acral	5 (5.5)
	Desmoplastic	3 (3.3)

SD: standard deviation; TNM: tumor, node, metastasis – TNM staging system.

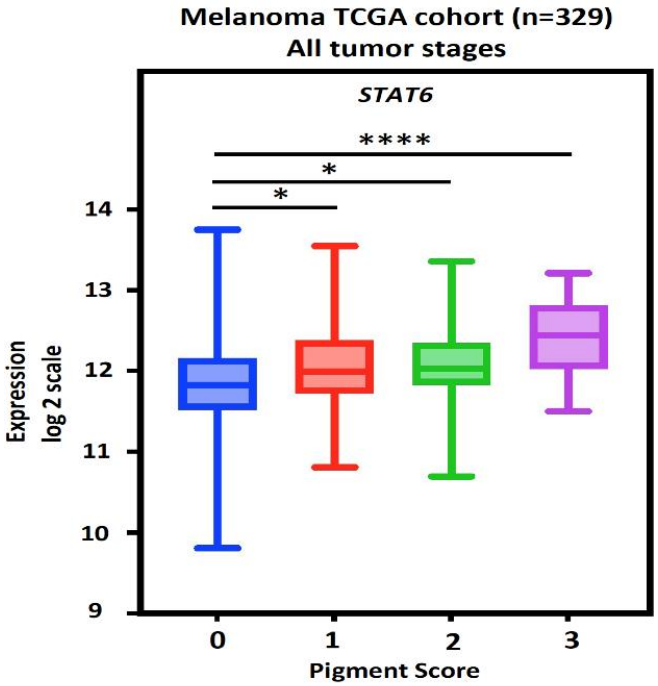

Figure 1 Transcriptional levels of the STAT6 gene according to pigmentation score (0 to 3) in melanomas. Expression levels were analysed using transcriptome datasets obtained from TCGA samples (n = 329). mRNA expression is shown in boxplot as log2-transformed signal intensity. Comparisons between subgroups were performed using the Kruskal-Wallis test followed by Tukey tests. Data are presented as median with whiskers representing the range from minimum to maximum. Statistical differences between all subgroups of pigmentation scores are shown in the panel; * $p < 0.05$, **** $p < 0.0001$ compared to non-pigmented tumors.

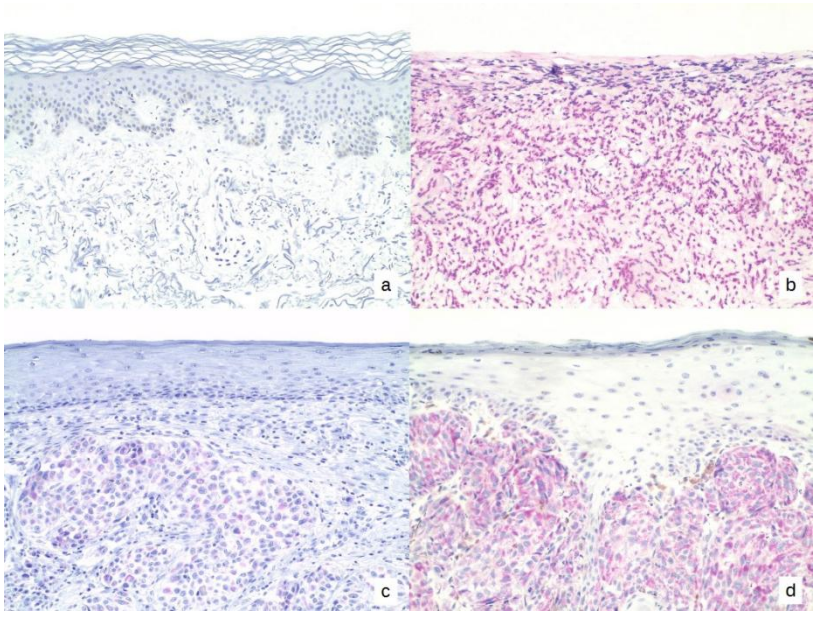

Figure 2 Images of representative samples of immunohistochemical expression of STAT6. (a) absence of expression in normal skin keratinocytes; (b) strong and predominantly nuclear expression in solitary fibrous tumor; (c) weak and predominantly cytoplasmic expression in a melanoma specimen with H score $\leqslant$ 85; (d) strong and predominantly cytoplasmic expression in a melanoma sample with H > 85. Magnification: 200 ×.

4 Discussion

In this study, we found that transcriptional levels of

STAT6, as well as STAT6 protein content, increases significantly in melanoma with higher melanin pigmentation scores. Immunohistochemical analysis showed both cytoplasmic and nuclear expression of STAT6 in the majority of analyzed samples, with expression levels correlating with tumor pigmentation. The overall agreement between global genomic data and immunohistochemical results supports the applicability of the findings, indicating that variations identified on a global scale can also be observed in Brazilian populations. These results highlight the importance of replicating international findings across different population contexts. Also, these findings suggest a possible particularly important role for STAT6 in the biology of more pigmented melanomas.

Protein expression of STAT6 was found in 28.6% of non-pigmented tumors, highlighting the complexity of its involvement in melanoma. STAT6 plays a multifunctional role in several cellular processes other than pigmentation, such as microenvironment modulation in non-pigmented tumors. For instance, IL-4 displays suppressive effects on experimental melanoma growth through p21-mediated activation of STAT6 [24], and IL-4 also inhibits melanogenesis of normal human melanocytes through activation of the JAK2-STAT6 pathway and regulation of the transcription and translation of melanogenesis-related genes, such as MITF [11], and there is activation of a DNA binding activity of STAT6 in B16F10 melanoma tumors from tissues of IL-4-overexpressing transgenic mice compared to controls [24].

Several studies have reported varying levels of expression of JAK-STAT pathway genes in breast, ovarian, lung, brain and colorectal cancer, which may be associated with different prognoses [16,18]. Expression of STAT1/2/3/5A/6 in gliomas was found to be correlated with mutations in the isocitrate dehydrogenase (IDH) gene [25]. IL-4/STAT6 signaling in CD11b+ cells promote the progression of lung cancer, suggesting that STAT6 could be a potential target for the prevention and treatment of lung cancer [26].

One previous study on cutaneous melanoma using TCGA data found that low levels of STAT6 expression were associated with a favorable prognosis, suggesting that this gene could serve as a biomarker [20]. The present study indicates an association between STAT6 gene and protein expression with increased melanoma pigmentation. In cutaneous melanoma, less tumor pigmentation may be associated with poorer prognosis [27], whereas, in uveal melanomas, heavy pigmentation is often associated with poorer prognosis [28]. Together with the present results linking STAT6 with pigmentation, this evidence indicates possible opposite roles of STAT6 in melanoma malignancy, depending on the tumor type.

Our study has several limitations, including its retrospective design, small number of samples for the immunohistochemical analysis, racial homogeneity in patient populations, and lack of control group for treatment.

5 Conclusion

Increased levels of STAT6 is related to higher levels of pigmentation in melanoma tumors, which may impact patient prognosis. Further research should explore the role of STAT6 in regulatory processes related to melanogenesis or the pigmented tumor microenvironment.

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Conflicts of Interest

The author of this article, Rafael Roesler, is a member of the editorial office of this journal. All procedures

during the editorial review process were conducted strictly in accordance with the journal's policies, and the author was not involved in handling any part of the process.

Author Contributions

K.M.P.A.C.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Roles/Writing - original draft, Writing - review & editing. B.L.S.: Roles/Writing - original draft, Writing - review & editing. J.S.: Project administration, Writing - review & editing. R.R.: Conceptualization, Roles/Writing - original draft, Writing - review & editing, Funding acquisition. H.F.J.: Conceptualization, Data curation, Resources. R.B.: Data curation, Investigation. P.H.C.F.: Data curation, Funding acquisition, Resources, Supervision, Writing - review & editing. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

This study was approved by the institutional Research Ethics Committee (approval number 4.037.886) and the study was conducted in accordance with the guidelines described in Resolution 466/2012 of the Brazilian National Health Council.

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Availability of Data and Materials

The dataset used for gene expression analysis is available through the Cancer Genome Atlas (TCGA) data portal (https://tcga-data.nci.nih.gov/tcga). Other data and materials are available upon request to the corresponding author.

Supplementary Materials

Not applicable.

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