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Usefulness of IMP3 and FOXP3 to predict metastasis of cutaneous melanomas

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Abstract

Background: Melanoma still has considerable mortality in spite of improvements in diagnosis and treatment. Unfortunately, current diagnostic procedures cannot predict precisely its biological behavior, what urges specialists in searching new better biomarkers of lousy prognosis. The objective of the study was to evaluate IMP3 and FOXP3 expression in primary skin melanoma lesions and to correlate with the presence of metastasis.

Methods: A retrospective cohort study analyzed 112 patients diagnosed with Melanoma, from 2003 to 2011, from a public health service. Samples from the primary lesion were analyzed by two pathologists and one dermatologist to ensure histological subtype, Breslow, the presence of ulceration, mitosis and histological regression. From the species stored, FOXP3 and IMP3 immunohistochemistry staining were performed. Demographic, clinical and evolution aspects of the patients were obtained from records, in the year of 2015. It was considered statistically significant when p -value < 0.05 .

Results: The majority of specimens had 25% or fewer cells stained with FOXP3 or IMP3. Their positivity could not be related to the occurrence of metastasis ($p = 0.947$ and $p = 0.936$, respectively).

Conclusion: There is no evidence of benefit in using IMP3 or FOXP3 as prognostic markers in primary melanomas in our population.

Keywords: Melanoma, Dermatopathology, Prognosis, Survival rate, Neoplasm metastasis

Background

In the United States, the frequency of melanoma has been increasing from 15 to 21.6 cases per 100,000 inhabitants representing an increase of 1.4% per year. Unfortunately, mortality remains stable. When it is confined to the epidermis (“in situ”), the survival rate in 5 years is near 98.3% (SEER Cancer Statistics Review 2017). When it is metastatic, the survival rate is below 18% (SEER Cancer Statistics Review 2017). Therefore, an early diagnosis is required because melanoma is a potentially curable lesion in initial steps.

Since 1969, melanoma has been divided into clinical and histopathological subtypes. There are four subtypes more frequent: Superficial Spreading (SS), Acral (Ac), Nodular (Nd) and Lentigo Maligna (LM) (de Vries et al. 2006).

The inability to completely predict the risk of developing metastasis is a significant barrier to the development of treatments with more efficiency (Albreski and Sloan 2009). Despite the improvement in follow-up and treatment, there are cases which seem to ignore the rules: thin melanomas with aggressive metastasis and thick lesions with prolonged disease-free survival (Albreski and Sloan 2009).

An immunohistochemistry marker named IMP-3 (Insulin-like growth factor-II messenger RNA (mRNA)-binding protein-3) is expressed by embryonic tissues and some neoplasms and play a role in post-transcriptional modulation of oncogenes related to cell proliferation, metastasis, chemotherapy resistance and survival. Most studies report it is positive in melanocytes cytoplasm in malignant and metastatic lesions (Pryor et al. 2008; Sheen et al. 2015).

Another subject of interest concerning escape mechanisms of melanoma from host defense is the expression of FOXP3 (Forkhead transcription factor). Its main function is to inhibit the response of TCD8 cells, which are

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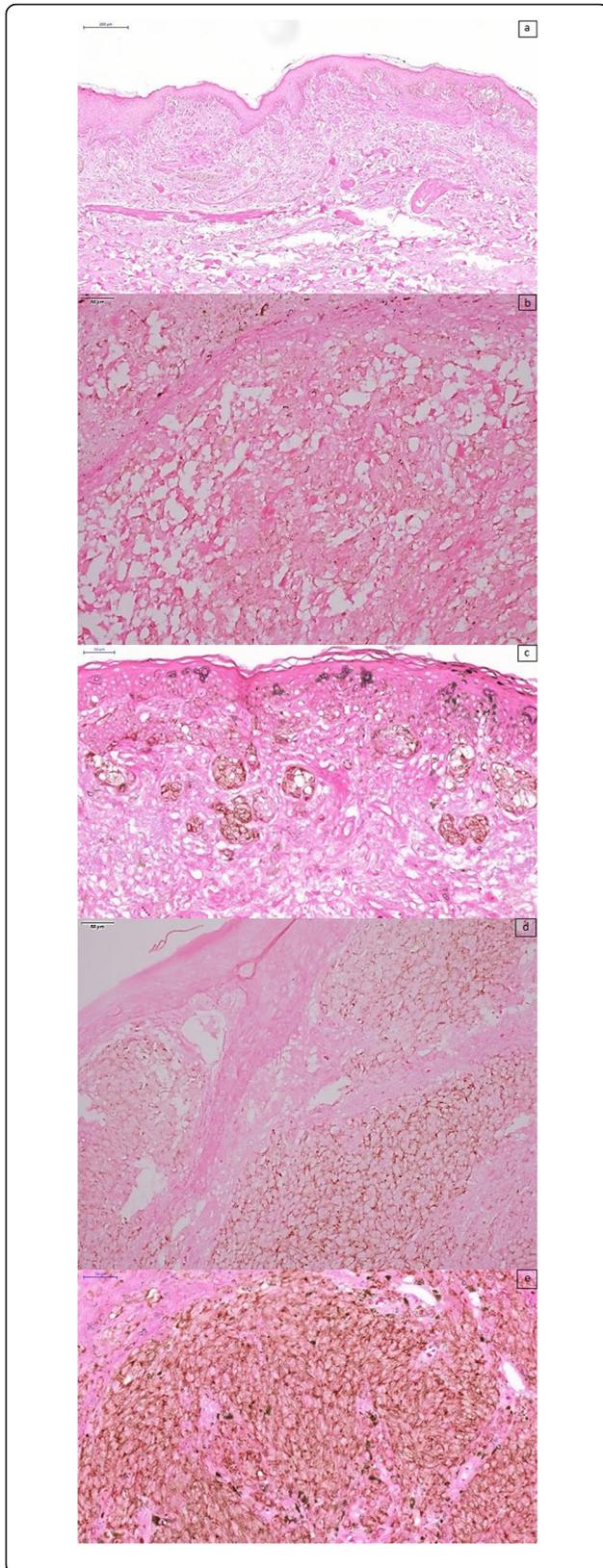


Fig. 1 Positivity to IMP3 immunostaining contra-colored by Giemsa, 400× Footnote: Score: zero (a), 1+ (b), 2+ (c), 3+ (d) and 4+ (e).

The positivity rate took into account the positivity in the total tumor area. Note that melanin appears as coarse black pigmentation

responsible for immune defense against tumors. It is mainly a nuclear marker of lymphocytes CD4 CD25^{high} but it can also be expressed by melanoma cells (Niu et al. 2011).

The purpose of this study is to evaluate IMP3 and FOXP3 expression in primary skin melanoma lesions and to correlate this presence with metastasis. This method is less expensive than molecular analysis, and it would be useful in prognostic markers leading to a better patients' follow-up and treatment.

Methods

The study included all patients diagnosed with Melanoma from Dermatology and Pathology services, from 2003 to 2011.

The study was approved by Institutional Review Board approval (Registration number: 33405714.1.0000.5411/2014) and informed consent was spared.

This retrospective cohort study analyzed records in December 2015, looking for epidemiologic and clinical variables: gender, age, lesion diameter, evolution to metastasis, death to melanoma or other diseases. The follow-up interval varied from 4 to 12 years.

The slides stained with Hematoxylin and Eosin were reviewed by two dermatopathologists and one dermatologist and a consensus was obtained in a multi-head microscope to verify: histological subtype, Breslow, the presence of ulceration, mitosis and histological regression.

Specimens fixed in 10% buffered formalin and embedded in paraffin were submitted to immunohistochemical staining of IMP3 and FOXP3.

Patients who had missed follow-up ($n = 56$), those whose data were incomplete ($n = 7$), cases of unclassified melanoma ($n = 3$), cases which histologic specimen was not available or not enough for new immunohistochemistry slides ($n = 17$) were excluded.

The samples were submitted to standard immunohistochemistry protocol through pt-LINK[®] and AutoStainer[®] equipment, in briefly, step-by-step procedures: antigen retrieval, blocking of endogenous peroxidase, incubation of conjugated antibodies and exposure through chromogen 3,3-diaminobenzidine (DAB – DAKO). Giemsa was used as contra-staining with the purpose of better discrimination between IMP3 and melanin.

Since there is no consensus on score of immunohistochemistry in IMP3 and FOXP3, we have established a stratification system which was applied to both markers. Those patients who had no cells marked in immunohistochemistry through visual analysis were graduated in 0, followed by 0 to 5% of positive cells (1+), 6 to 25% (2+), 26 to 50% (3+) and 50 to 100% positive cells were

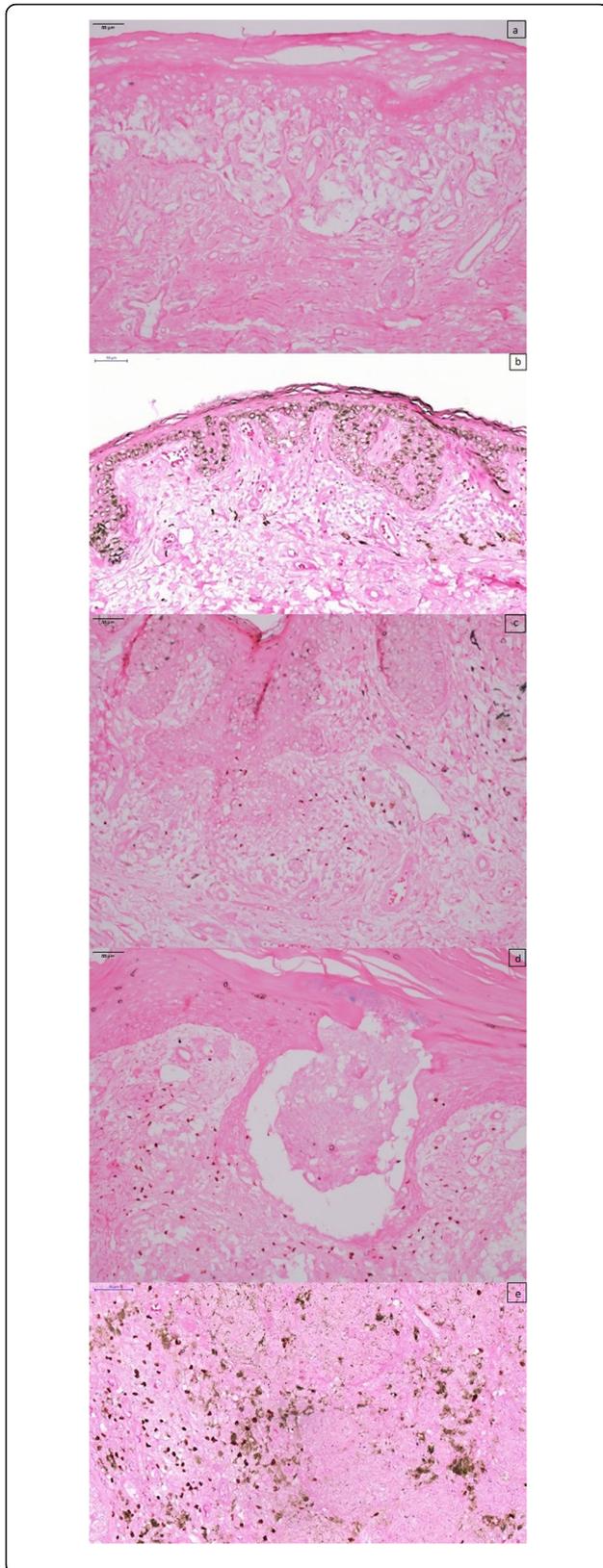


Fig. 2 Positivity to FOXP3 immunostaining contra-colored by Giemsa, 400× Footnote: Score: zero (a), 1+ (b), 2+ (c), 3+ (d) and 4+ (e). The positivity rate took into account the positivity in the total tumor area. Note that melanin appears as coarse black pigmentation

graduated in 4+. Cases that had score 3+ or higher were considered “positive” for statistical analysis.

Data was submitted to Cox’s regression analysis. A ROC’s curve was assessed to establish age’s cut off. It was considered statistically significant when CI was > 95% or when p - value was < 0.05.

Results

All melanomas diagnosed between the years of 2003 and 2011 totaled 195 cases. After applying excluding criteria, 112 patients have submitted to IMP-3 and FOX P3 immunohistochemistry staining (Figs. 1 and 2). All metastasis, local or distant were considered as the same denouement event.

In this sample, 47.3% of the patients were females, which was not associated with metastasis ($p = 0.495$) (Table 1). Age after 61.5 years old (established by a ROC curve application) was associated with metastasis, comparing to the younger group ($p = 0.032$).

Subtypes frequency was similar to the previously described in literature [2,3]: 64.3% of SS, 15.2% of Ac, 10.7% of Nd and 9.8% of LM. Our sample had thin Breslow (0.7, ranging from 0 to 25 mm), low frequency of mitosis (median of 0, varying from 0 to 50), ulceration (30.4%) and progression to metastasis (24.1%) what represents that most cancers were diagnosed early.

Nodular subtype was the one which had more association with metastasis (OR = 9.00), but all subtypes had a higher risk in comparison with superficial spreading.

Breslow, ulceration and mitosis were associated with metastasis. The OR for 1 to 4 mm of Breslow was 8.33, and for Breslow > 4 mm the OR was 14.44 ($p < 0.001$). (Table 1) When there were more than five mitoses, the OR was 15.65 against 13.44 when there were fewer mitoses. Ulceration was related to metastasis with an OR of 5.44, $p < 0.001$.

Near half of the patients showed histological regression (49.1%). Regression was inversely associated with metastasis (OR = 0.36, $p = 0.021$).

The majority of cases had 25% or less positivity of both IMP3 and FOXP3. Their positivity could not be related to metastasis ($p = 0.947$ and $p = 0.936$, respectively). It was not possible to analyze the event “death” because its number was very low.

Discussion

The main finding of that study was the uselessness of IMP3 an FOXP3 to predict melanoma metastasis in all subtypes, in the studied sample.

Table 1 Metastasis' risk related to clinical and histopathological variables

Variables		Without metastasis	With metastasis	OR (CI95%)	<i>p</i>
Gender	Female	42(79%)	11(21%)	1.00	
	Male	43(73%)	16(27%)	1.30 (0.60–2.81)	0.495
Age*	≤61.5	55(85%)	10(15%)	1.00	
	>61.5	30(64%)	17(36%)	2.35 (1.07–5.13)	0.032
Subtype*	SS	66(92%)	6(8%)	1.00	
	Ac	9(53%)	8(47%)	5.65 (1.95–16.27)	0.001
	Nd	3(25%)	9(75%)	9.00 (3.20–25.28)	<0.001
Breslow*	LM	7(64%)	4(36%)	4.36 (1.23–15.46)	0.022
	[0–1]	62(95%)	3(5%)	1.00	
	(1–4]	16(62%)	10(38%)	8.33 (2.29–30.27)	0.001
Ulceration*	>4	7(33%)	14(67%)	14.44 (4.15–50.26)	<0.001
	Absence	70(90%)	8(10%)	1.00	
Mitosis*	Presence	15(44%)	19(56%)	5.44 (2.38–12.44)	<0.001
	0	58(97%)	2(3%)	1.00	
IMP3	[1–5]	16(55%)	13(45%)	13.44 (3.03–59.59)	0.001
	>5	11(48%)	12(52%)	15.65 (3.50–69.93)	0.001
	≤2+	76(76%)	24(24%)	1.00	
FOXP3	>2+	9(75%)	3(25%)	1.04 (0.31–3.45)	0.947
	≤2+	75(76%)	24(24%)	1.00	
Regression*	>2+	10(77%)	3(23%)	0.95 (0.28–3.16)	0.936
	Absence	37(65%)	20(35%)	1.00	
	Presence	48(87%)	7(12%)	0.36 (0.15–0.85)	0.021

Cox's Regression model. *p* < 0.05*

*marks variables that showed statistical significance

There are few studies of the use of IMP3 immunostaining in melanoma. However, it is considered positive if present in at least 10% of melanocytes by direct observation (Pryor et al. 2008; Sheen et al. 2015). Some demonstrated that it was negative in common and dysplastic nevi, slightly positive in thin melanomas and Spitz nevi and strongly positive in metastatic melanomas (Pryor et al. 2008). Others have suggested a worse prognosis in IMP3 positive cancers (Sheen et al. 2015). Nevertheless, Chokoeva et al. (2015) have shown positivity in only 40% of melanomas and 20% of dysplastic nevi, advocating that it is a weak prognostic marker.

In this study, approximately 10% of the melanomas had more than 25% positive cells. Five instances of superficial spreading melanoma, four instances of acral melanoma, one instance of nodular melanoma and one instance of lentigo maligna melanoma showed positivity of 2+. The positivity rate would be near 20%, if 2+ group had been considered positive too. Our study did not show a correlation between IMP3 and metastasis (*p* = 0.947), and resetting the cut off value did not render the analysis significant (data not available in tables). Only three out of the metastatic melanomas, which

means only 11% of metastatic melanomas (3/27), had more than 25% of positive cells.

Several studies in animals indicated positive correlation between CD4 + CD25 + FOXP3+ cells presence and tumor progression. Murine melanomas had a delay in time to a visible lesion, decrease in tumor weight and increase in survival time when FOXP3 was inhibited (Franco-Molina et al. 2016). On the other hand, Ladányi et al. (2010) and Hillen et al. (2008) did not associated FOXP3 presence with a worse prognosis. This study also did not show its correlation with metastasis (*p* = 0.936). Most of the positive cases were superficial spreading subtype (7 cases), followed by lentigo maligna (3 cases), nodular (2 cases) and acral subtype (1 case). Our study analyzed the primary lesion, in agreement with other studies (Ladányi et al. 2010; Hillen et al. 2008). FOXP3 could be useful in analyzing metastasis instead of primary lesions.

The low frequency of denouement events, mainly death, was the main limitation of the study. Therefore, the study collected all possible patients in 9 years, with the minimum follow-up of 4 years and a maximum of 12 years which represents an excellent portrait of our population.

Conclusions

In conclusion, there is no evidence of benefit in the use of IMP3 or FOXP3 as prognosis markers in primary melanomas in our population. Clinical and pathological features as mitoses, Breslow, ulceration and histological subtype were associated with metastasis in accordance with previous studies. Regression was inversely associated with metastasis. More investigative studies are necessary to reinforce those statements. Pathologists and dermatologists could be cautious before accomplishing new prognostic markers in their routine.

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Availability of data and materials

Data and Materials available for requests.

Authors' contributions

All the authors participated of data collecting, statistics analyses, writing and review. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by Institutional Review Board approval (Registration number: 33405714.1.0000,5411/2014).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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