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Role of CALLA/CD10 Expression in Progression of Melanocytic Tumors: A Study in Egypt

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Abstract

BACKGROUND: Although most of melanocytic lesions can be diagnosed using morphology, there is a significant subset of lesions that are difficult to diagnose. These are a source of anxiety for patients, clinicians, and pathologists. This arouses the possible benefits of using ancillary techniques to solve this problem. CD10 is a zinc-dependent metalloproteinase, its expression is known to be associated with biological aggressiveness in various malignancies.

AIM: This research observes the efficacy of CD10 in the progression of melanocytic tumors as well as the differential diagnosis between nevus and melanoma.

METHODS: The material of this study included 49 paraffin blocks of Egyptian melanocytic tumors. CD10 expression either membranous and/or cytoplasmic in tumor cells was considered positive and scored, based on the percentage of cells stained and compared to Ki67 expression as a prognostic marker.

RESULTS: In benign melanocytic nevi, only 16.7% of cases showed positive expression, all were + 1 score, compared to 82.6% of melanoma cases, mostly +1 score followed by +3 score and finally +2 score. The difference in CD10 expression among melanocytic tumors showed a highly statistically significant correlation between nevus and melanoma cases as well as in Spitz nevi versus other nevi. Another highly statistically significant correlation was observed between CD10 expression and both Ki67 expression and ulceration.

CONCLUSION: CD10 expression was significantly higher expressed in melanomas rather than nevi with highly statistically significant positive relation with Ki67 and ulcer formation which supports its role as a potential biomarker in the development of malignant melanoma and marker of aggression.

Introduction

Melanocytic tumors are common in routine surgical pathology, especially in hot countries like Egypt, where people are subjected to ultraviolet rays of the sun everyday. Benign melanocytic nevi (BMN) are localized benign melanocyte abnormalities straddling the fence between malformations and neoplasia, while melanoma is simply the malignant neoplasm of melanocytes. Cutaneous melanoma (CM) is steadily increasing in incidence more than any other malignancy (Berwick *et al*., 2016) [1]. In Egypt, CM accounts for 4.7% of all cutaneous malignancies according to the National Cancer Institute (Mokhtar *et al*., 2016) [2].

Although most of melanocytic lesions can be confidently diagnosed using well-established morphologic criteria, there is a significant subset of lesions that can be difficult to diagnose. These can be a source of anxiety for patients, clinicians, as well as pathologists, moreover, the potential consequences of a missed diagnosis of melanoma are serious. Small incomplete biopsies of pigmented lesions are a daily source of frustration for any pathologist (Nathan *et al*., 2019) [3]. This arouses the possible benefits of using different ancillary techniques to solve this problem.

CD10 (common acute lymphoblastic antigen) is a zinc-dependent metalloproteinase, which is commonly expressed in bone marrow lymphoid stem cells, pro-B lymphoblasts, various lymphoma subtypes, renal cell carcinoma, and endometrial stromal sarcomas. Its expression is known to be associated with biological aggressiveness in various epithelial malignancies (Ashish *et al*., 2019) [4]. It also plays an important role in the pathogenesis of prostate cancer as well as its increased and altered expression is especially seen in high Gleason scores, lymph nodes, and bone metastasis (Lalit *et al*., 2019) [5].

This research observes the efficacy and role of immunohistochemical expression of CD10 in studying the progression of melanocytic tumors, by comparing it to the different prognostic factors including Ki67, as well as its possible application in the differential diagnosis between BMN and melanoma. Although we are not the first researchers to investigate this topic, to our knowledge, no researches have addressed Egyptian patients before to see whether our results match other nations or not, which we believe is very important to our society.

Materials and Methods

The material of this study included 49 formalin-fixed, paraffin-embedded tissue blocks of melanocytic tumors from Egyptian patients, who underwent either excision or incisional biopsy, collected from archives of Pathology department, Kasr AL-Ainy Hospital, Faculty of Medicine, Cairo University. The data collected from the pathology requisition sheets enclosed with the specimens included age at diagnosis, sex, and tumor size.

Each paraffin block was recut by rotatory microtome at 4 μ thickness then mounted on a glass slide and stained by hematoxylin and eosin for histopathological reassessment and on a positively charged slide for immunohistochemical staining. The tumor sections were also examined for tumor thickness and presence of surface ulceration. Melanin bleaching with warm diluted hydrogen peroxide was performed when needed.

Immunohistochemical staining

Heat-mediated antigen retrieval was performed with citrate buffer pH 6 in automated water bath (Dako PT link, PT101). Sections were stained for antiCD10 antibody (mouse monoclonal IgG1 CD10, clone 56C6), and anti-Ki67 antibody (mouse monoclonal IgG1 Ki67, clone GM010) obtained from Genemed Biotechnologies (458 Carlton Ct, South San Francisco, CA, USA) and used at a dilution of 1:25. Immunohistochemical staining was performed in an autostainer (Dako autostainer link 48) using a polymer-based detection system (Dako EnVision™ FLEX, K8000). Diaminobenzidine (DAB) was used as chromogen and hematoxylin as counterstain. Sections of normal renal tissue and tonsil were used as a positive control for CD10 and Ki67, respectively.

Immunohistochemical evaluation

All sections were screened to explore the areas with well-preserved tissue architecture and cell morphology for immunoreactivity scoring. Areas with tissue morphology disruption due to processing or melanin bleaching were discarded in the analysis.

CD10 expression either membranous and/or cytoplasmic in tumor cells was considered positive and scored, based on the percentage of cells stained, which was determined in at least five areas at 400-fold magnification, as follows:

- Score 3+: ≥50% of CD10-positive tumor cells
- Score 2+: 10–50% of CD10-positive tumor cells
- Score 1+: <10% of CD10-positive tumor cells (Hoshikawa *et al*., 2015) [6].

Ki67 immunostaining was evaluated in the fields of maximal staining using labeling index (LI). LI was the number of positive tumor nuclei expressed as a percentage of the total number of tumor nuclei counted. A total of at least 1000 nuclei were counted in each case. LI cutoff value of 20 was used to group cases into two groups:

- LI <20
- LI >20 (Ashish *et al*., 2019) [4].

Statistcal analysis

Microsoft Excel 2010 was used for data entry and the (Statistical Package for the Social Sciences software, version 24, Chicago, IL, USA) was used for data analysis. Simple descriptive statistics (arithmetic mean ± standard deviation, median, and range) were used for normal quantitative data, and frequencies used for qualitative data. Bivariate relationship was displayed in cross-tabulations and comparison of proportions was made using the Chi-square and Fisher's exact tests wherever appropriate. T-independent and ANOVA test were used to compare normally distributed quantitative data. $p < 0.05$ was considered statistically significant.

Microscopic photos were captured using a digital camera attached to an Olympus microscope BX 51.

This study was approved by the Ethical Committee of Faculty of Medicine, Cairo University.

Results

This study is a preliminary study including 49 Egyptian cases of melanocytic tumors, distributed as 24 cases of BMN, 23 CM, as well as 1 case of dysplastic nevi and 1 case of *in situ* lesion. The latter two cases were not satisfactory to establish statistical results, so were discarded.

Clinicopathological variables

Mean age in BMN was 25 years while in melanoma cases, it was 60 years. As regards sex, our BMN cases showed predominance of female sex while our melanoma cases showed equal distribution among both sexes. BMN cases were subtyped as 45.8% (11 cases) dermal, 37.5% (9 cases) compound, and 16.6% (4 cases) Spitz nevi. Twenty-one cases of our melanoma cases were primary while the remaining two cases were metastatic. Nodular melanoma was the most common histologic subtype (85.7%). Regarding the largest tumor diameter, in BMN cases, it ranged between 0.1 cm and 19 cm with mean of 3.8 cm, while the largest diameter of the studied 21 melanoma cases (could not be assessed in two cases) ranged between 0.5 cm and 7 cm with mean of 2.7 cm. Tumor thickness in the studied BMN cases ranged between 0.2 cm and 2 cm with mean of 0.9 cm. Tumor thickness in the studied 21 melanoma cases (thickness could not be assessed in two cases)

ranged between 0.4 cm and 3.5 cm with mean of 1.57 cm. The tumor thickness was then classified into two groups for statistical reasons; ≤0.2 cm and >0.2 cm, accordingly, all studied melanoma cases' thickness was >0.2 cm, compared to 79.2% only of the studied BMN cases. Concerning ulceration, none of the BMN cases showed ulceration. Most of studied melanoma cases (19 cases, 82.6%) presented with ulcerations.

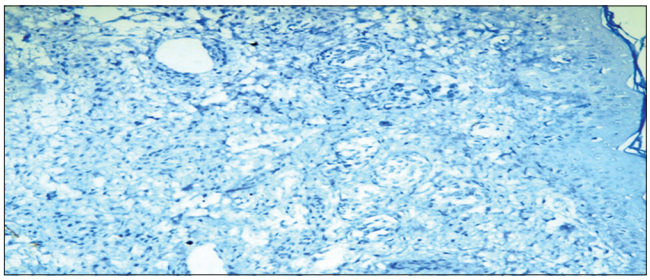

Figure 1: This is a case of dermal benign melanocytic nevus showing negative CD10 immunoexpression (CD10 × 200 original power)

CD10 expression

In BMN, 20 (83.3%) cases were CD10 negative (Figure 1), while only 4 cases (16.7%) showed positive CD10 expression in tumor cells, all were + 1 score (Figure 2).

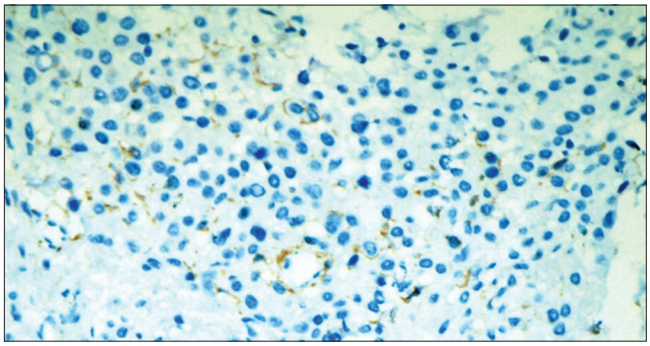

Figure 2: This is a case of compound benign melanocytic nevus showing positive CD10 immunoexpression in >10% of tumor cells representing score +1 (CD10 × 400 original power)

In melanoma, 82.6% of cases (19 cases) showed positive CD10 expression in tumor cells mostly +1 score (11 cases, 57.9%) followed by +3 score (5 cases, 26.3%) (Figure 3) and finally +2 score (3 cases, 15.8%). \

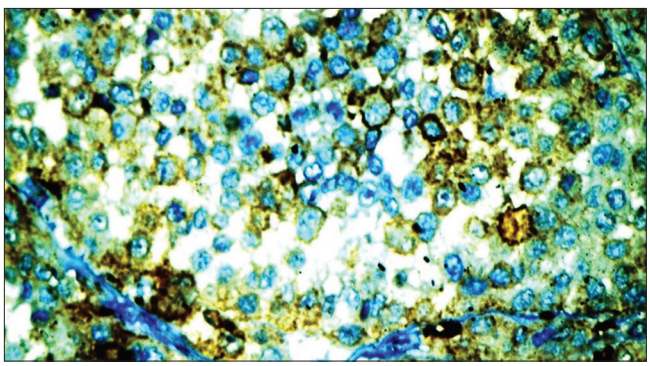

Figure 3: A case of melanoma showing positive CD10 immunoexpression in >50% of tumor cells representing score +3 (CD10 × 400 original power)

Ki 67 expression

All BMN cases showed Ki67 LI <20, while all melanoma cases showed Ki67 LI >20 (Figure 4).

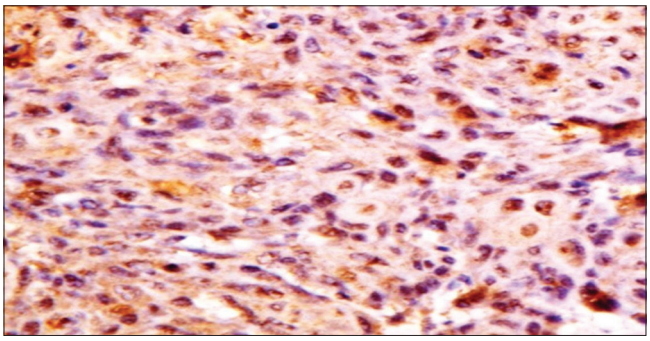

Figure 4: A case of melanoma showing Ki67 LI >20 (Ki67 × 400 original power)

Correlation between CD10 expression and the statistically significant clinicopathological variables in both BMN and melanoma are represented in a table form.

A highly statistically significant positive correlation was found between CD10 expression and Ki67 expression as mentioned in Table 1, 83.3% of cases showing Ki67 LI <20 were negative to CD10. On the other hand, 82.6% of cases showing Ki67 LI >20 were CD10 positive. We also observed that all melanoma cases showing ulceration positively expressed CD10. On the other hand, all melanoma cases lacking ulceration were negative to CD10 expression. These results were statistically highly significant (p < 0.001).

Table 1: Correlation between CD10 expression and the statistically significant clinicopathological variables in BMN and melanoma cases

Clinicopathological variables	CD10 expression (%)					p value
	Negative	Positive				
		+1	+2	+3	Total of+cases	
BMN versus melanoma						
BMN	20 (83.3)	4 (100)	0 (0)	0 (0)	4 (100)	<0.001
Melanoma	4 (17.4)	11 (57.9)	3 (15.8)	5 (26.3)	19 (100)	
Melanoma types						
Primary	3 (14.3)	17 (80.9)	0 (0)	1 (4.8)	19 (100)	0.006
Metastatic	1 (50)	0 (0)	1 (100)	0 (0)		
BMN types						
Dermal and compound	18 (90)	2 (100)	0 (0)	0 (0)	4 (100)	0.05
Spitz	2 (50)	2 (100)	0 (0)	0 (0)		
Ki67 LI						
<20	20 (83.3)	4 (100)	0 (0)	0 (0)	4 (100)	<0.001
>20	4 (17.4)	11 (57.9)	3 (15.8)	5 (26.3)	19 (100)	

The age of our cases did not affect CD10 expression and the relation between them was statistically insignificant (p = 0.65). The same results apply to sex (p = 0.27). Although CD10 expression was directly proportionate to melanoma largest diameter, the relation between them was statistically insignificant (p = 0.75). Involvement of deep margin of resected melanomas was not related to CD10 expression and had statistically insignificant correlation (p = 0.73).

The application of CD10 expression in melanocytic tumors showed sensitivity of 82.6%, specificity of 83.3%, positive predictive value of 82.6%, and negative predictive value of 83.3%.

Discussion

In our research, we studied 49 Egyptian melanocytic tumors distributed as 24 BMN, 23 melanomas 1 dysplastic and 1 *in situ*, which were both disregarded for statistical reasons. Most of our studied BMN cases were CD10 negative (20 cases, 83.3%), all the remaining positive cases showed +1 score. In agreement with our results, Hoshikawa *et al*. [6], 2015, studied 50 BMN cases all were CD10 negative and Oba *et al*. [7], 2011, who studied 40 BMN cases showing positive CD10 staining in only 10%.

The majority of our studied melanoma cases showed positive staining (19 cases, 82.6%) mostly +1 score (11 cases, 57.9%) followed by +3 score (5 cases, 26.3%) and finally +2 score (3 cases, 15.8%). These results, although similar to but, were much higher than those stated by Oba *et al*. [7], 2011, who like us found that CD10 positivity was seen in most of melanoma cases but with lower percentage (53%). Unlike our results, less CD10 prevalence was reported by the studies done by Hoshikawa *et al*. [6], 2015 (47%), and Bilalovic *et al*. [8], 2004 (22%). The higher CD10 expression in our study could be related to our smaller sample size.

Concerning subtypes, most of the BMN cases were dermal and compound with 90% showing negative immunostaining and the remaining 10% showing +1 score, while the four included Spitz nevi cases two were negative and two showed +1 score, declaring a statistically significant increase in CD10 expression in Spitz nevus rather than other types ($p = 0.05$). As for melanoma, primary melanoma showed positive CD10 predominance mostly of score +1, compared to metastatic melanoma which showed CD10 expression in half of cases only. Our results were highly significant ($p = 0.006$). However, the studies performed by Kanitakis *et al*. [9], 2002, Velazquez *et al*. [10], 2007, and Thomas-Pfaab *et al*. [11], 2013, illustrated more CD10 expression in metastatic rather than primary melanomas.

The difference in CD10 expression among melanocytic tumors in our research showed a statistically significant correlation either between BMN and melanoma cases ($p < 0.001$) or between primary versus metastatic melanoma ($p = 0.006$) or in Spitz nevi versus other more common nevi ($p = 0.05$) and this supports the role of CD10 in the progression of melanocytic tumors as well as its malignant potential in melanoma. Hoshikawa *et al*. [6], 2015, results were similar to ours concerning CD10 expression in melanoma versus BMN. This could be attributed to CD10 upregulation in more aggressive tumor types aiding their oncogenic properties, progression, and invasion. Fortunately, this is consistent with the purpose of our study to prove that CD10 could stand as an independent prognostic factor in melanocytic tumors denoting worse prognosis.

Adding to this, Ki67 expression, which was found to be more expressed in melanomas rather than BMN, also upregulated with CD10 expression in a highly statistical manner. These results agreed with Bilalovic *et al*. [8], 2004, as well as Hoshikawa *et al*., 2015 [6]. Another highly statistically significant relation was observed between CD10 expression and ulceration in melanoma cases emphasizing its poor prognostic value. To the best of our knowledge, this parameter has not been compared before.

Concerning the relation between CD10 expression and the patient's other different clinicopathological variables, such as age, sex, tumor thickness, largest diameter, and deep margin involvement, of both BMN and melanomas, our study could not achieve any statistically significant correlation. To date, these correlations have not been considered in other comparative studies. About the accuracy of this study, by comparing our results in melanomas versus BMN, this study was 82.6% sensitive, 83.3% specific, with positive predictive value of 82.6% and negative predictive value of 83.3%. Close results were obtained by Hoshikawa *et al*., 2015 [6], concerning the specificity, positive predictive value, and negative predictive value (100%, 100%, and 73%, respectively) but different regarding the sensitivity (47%) which can be due to different sample sizes and tumor subtyping.

We conclude that CD10 expression was significantly higher in melanomas rather than BMN with high sensitivity and specificity, besides, it shows positive correlation with Ki67 and ulceration, which supports its role as a potential biomarker in the development of malignant melanoma and as a marker of aggression. Thus, the use of CD10 immunohistochemical staining in pathological specimens of melanocytic tumors can have a prognostic value. Moreover, the application of CD10 as a diagnostic immunohistochemical marker in melanocytic tumors may be of value as it showed an acceptably high sensitivity and specificity in melanomas versus BMN. The results obtained from Egyptian patients agreed with most of the other global results except that we observed higher CD10 expression in primary versus metastatic melanomas unlike other nations. A limitation in our study was our inability to find more cases of dysplastic nevi and *in situ* lesions unfortunately. Other researchers are encouraged to continue in this field.

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material obtained in this study was collected in the form of archived paraffin blocks and clinical data were taken from pathology request sheets designated by numbers, therefore, no consent from patients was required. All steps of this research were approved by the ethical committee.

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