

Correlation between High Endothelial Vessels and Histopathological Features of Different Pigmented Lesions

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ABSTRACT: Purpose: Tumor infiltrating lymphocytes are playing an important role in cutaneous melanoma being a strong prognostic parameter. Our goal was to study the presence of high endothelial vessels in correlation with the histopathological features in different pigmented skin lesions. Material and methods: our study group included 60 patients (20 cases with dysplastic nevi, 20 thin melanoma and 20 thick melanoma). For each patient we noted epidemiological and clinico-pathological characteristics including: age, gender, anatomic sites, regression, Breslow thickness, mitoses, Clark level and lymphocytic infiltration. Using immunohistochemistry staining we identified the presence of high endothelial vessels in our groups. Results: the most common localization of primary melanoma was trunk 57,5%, followed by extremities 35% and head 7,5%. We found positive MECA-79 vessels in 67% of primary melanoma samples and in 30% of dysplastic nevi. Lymphocytic infiltration was present in 80% samples of dysplastic nevi and 75% of primary melanomas. Using Kruskal Wallis non-parametric test we found a positive association between MECA-79+ vessels and different anatomic sites ($p < 0,01$). We have also found a significant correlation between MECA-79+ vessels and the presence of regression in melanoma samples. In conclusion a better understanding of tumor microenvironment and mechanisms involved in anti-tumor response might play an important role in development of future melanoma therapeutic strategies.

KEY WORDS: melanoma, tumor infiltrating lymphocytes, high endothelial vessels, MECA-79, dysplastic nevi

Introduction

Although cutaneous melanoma represents less than 5% of the skin cancer is responsible for 80% of cancer deaths with this localization. [1]

Dysplastic nevi are described like as a precursor lesions for melanoma. Many studies have also shown that superficial spreading melanomas are found in histological contiguity with a dysplastic nevi. [2]

Tumor infiltrating lymphocytes are playing an important role, being a strong maker for the immune response in malignant melanoma. The migration of the lymphocytes occurs via high endothelial vessels (HEV), blood vessels that are generally found in the lymphoid structures. [3]

Recently, some studies reported the presence of HEVs in solid tumors, including melanoma. In light microscopy the endothelial cells of HEVs have a “plump” appearance, examination being surrounded by lymphocytes. This fact supports the hypothesis of their implication in lymphocytic migration. HEV endothelial cells express high levels of 6-sulfo sialyl Lewis X ligands recognized by the specific antibody MECA-79. [4]

In this study we have evaluated the potential correlations between the histopathological

features of three different skin lesions (dysplastic nevi, thin and thick cutaneous melanomas) and the detected number of HEV blood vessels (assessed by the expression levels of the MECA-79).

Materials and Methods

Samples

We have included formalin-fixed and paraffin-embedded tissue samples from a retrospective cohort of patients with dysplastic nevus and primary melanoma collected from 2002 to 2011 (at the Department of Pathology of the Hospital Clinico Universitario, Valencia). The work was conducted in accordance with the Declaration of Helsinki Principles and under the supervision of the Hospital Clinico Universitario Ethics Committee. We have analyzed a total of 60 samples, 20 of dysplastic nevi and 40 samples of primary cutaneous melanoma (20 samples of thin melanoma <1mm and 20 samples of thick melanoma >1mm) during a 10 year period (2002-2011). The included clinico-pathological characteristics were: age, gender, anatomic sites, regression, Breslow thickness, mitoses, Clark level, the lymphocytic infiltration (Table 1).

Table1. Characteristics of the patients included in the study

Characteristics	Dysplastic nevi	Primary melanoma
Gender		
Male	11	13
Female	9	27
Age		
≤ 60	18	18
> 60	2	22
Localization		
Extremity	3	14
Trunk	16	23
Head and neck	1	3
Clark		
I	-	3
II	-	7
III	-	15
IV	-	11
V	-	4
Ulceration		
Yes		10
No		30
Lymphocytic infiltration		
Yes	16	29
No	4	11
MECA-79 positive vessels		
Yes	12	26
No	8	14

Immunohistochemistry

The immunohistochemical study (Envision, ref. K5007, Dako, Denmark) was performed with HEV-specific mouse monoclonal antibody MECA-79.

Antigen retrieval was performed following the manufacturer recommendation.

Endogenous peroxidase activity was blocked by incubation in S2023 (Dako) for 10 min. The slides were incubated for 30 min. with primary antibody MECA-79 (final dilution 100 µg/ml, 30', at room temperature). Dilutions and washings were carried out with washing buffer (ref. S3006, Dako). The slides were then counterstained with hematoxylin. Adjacent control sections were incubated with monoclonal anti-sialyl-I antibody at the same concentrations and using the same procedure.

Evaluation of the immunohistochemistry was performed by two of the authors (CM and GA), having no knowledge of clinical or molecular data, using an Olympus BX40 light microscope, and LEICA DMD108 digital microscope (Leica Microsystems, Germany).

Statistical analysis

The statistical analysis was performed using the SPSS 17.0 software package.

The comparison between groups were analyzed by the two-tailed non-parametric Mann-Whitney U test and Kruskal-Wallis test. A p value of <0.05 was considered statistically significant.

Results

Of 60 patients, 60 % were women and 40% men. The most common localization of primary melanoma was trunk 57.5%, followed by extremities 35 % and head 7.5%. (Fig.1).

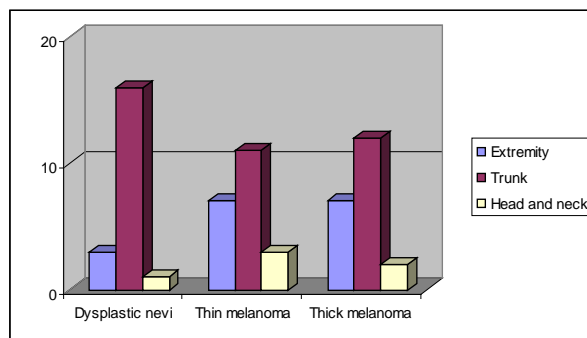


Fig.1. Localization of skin pigmented lesions included in the study

Lymphocytic infiltration was present in 80% sample of dysplastic nevus and 75% of primary melanomas.

The predominant Clark index was III for 15 samples, IV in 11 samples, II in 7 samples, V in 4 cases and I in 3 cases (Fig.2).

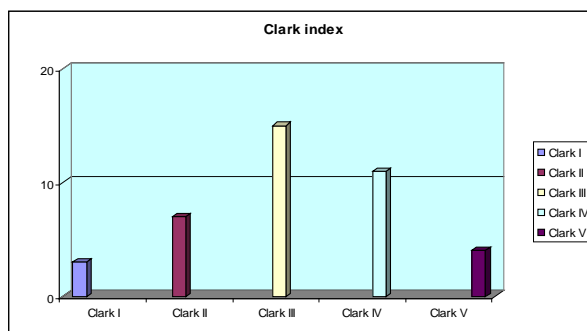


Fig.2. Distribution of melanoma samples by Clark index

Ulceration was present in 25% of melanoma samples. Area of regression was observed for 35% of the investigated melanoma samples. Positive MECA-79 vessels were found at 67% of primary melanoma samples (Fig. 3, 4) and 30% of dysplastic nevi (Fig. 5).

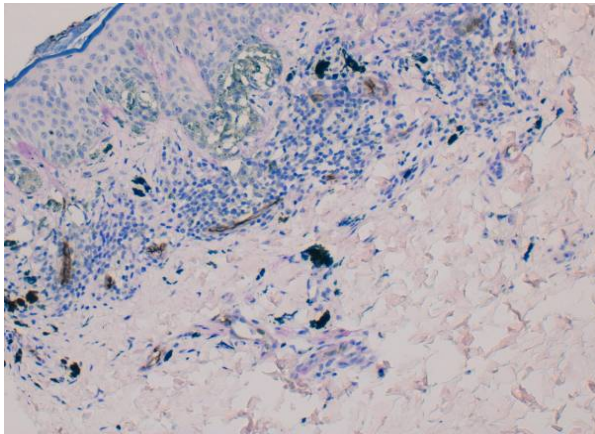


Fig.3. Dysplastic nevus, MECA-79 positive vessels, X20

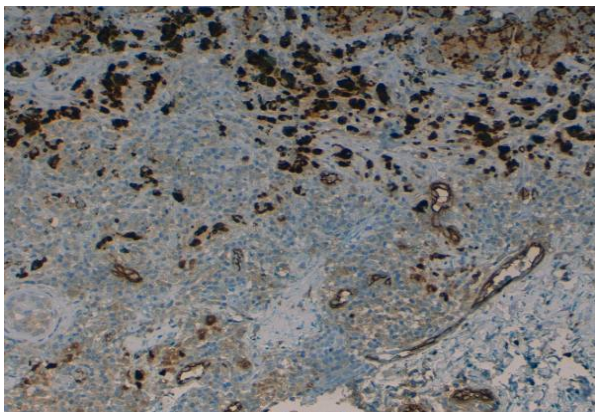


Fig.4. Thin cutaneous melanoma(<1mm), MECA-79 positive vessels, X20

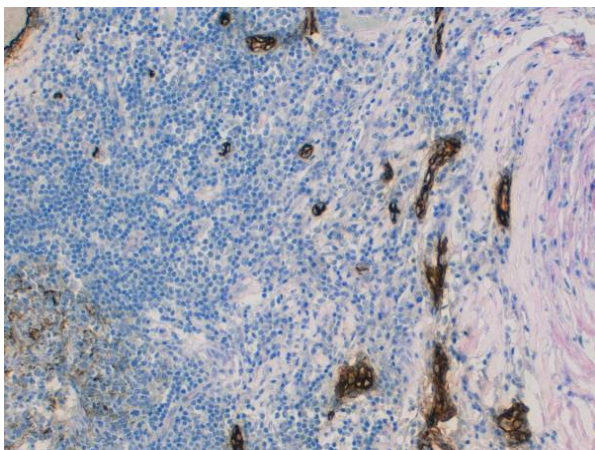


Fig.5. Thick melanomas (>1 mm), MECA-79 positive vessels, X20

Using Kruskal Wallis non-parametric test we found a positive association between high endothelial vessels and anatomic sites ($p < 0,01$). We noticed using Mann-Whitney test a significant correlation between MECA-79+vessels and the presence of regression in melanoma samples ($p < 0,05$).

Finally we found no significant association between the number of vessels and age, gender,

anatomic sites, Breslow thickness, ulceration, Clark level.

Discussion

Patients with multiple dysplastic nevi have an increased risk for development of melanoma and to make the distinguish from dysplastic nevi, melanoma sometimes can be challenging. [5]

Malignant melanoma is a neoplasm that more often tends to undergo regression. Regression is associated with dense lymphocytic infiltrates, the process starts with a dense lichenoid infiltrate of lymphocytes, and ends with fibrosis and/or melanosis within a thickened papillary dermis. [6,7]

High endothelial vessels are implicated in lymphocytic trafficking, recently some studies reported the presence of HEVs in solid tumors, including melanoma fact that sustain the implication of this type of vessels in anti-tumor response. [8]

In this study we have evaluated the potential correlations between the histopathological features of three different skin lesions (dysplastic nevi, thin and thick cutaneous melanomas) and the detected number of HEV blood vessels (assessed by the expression levels of the MECA-79).

We observed that the highest number vessels were found in thin melanoma samples (Fig. 6).

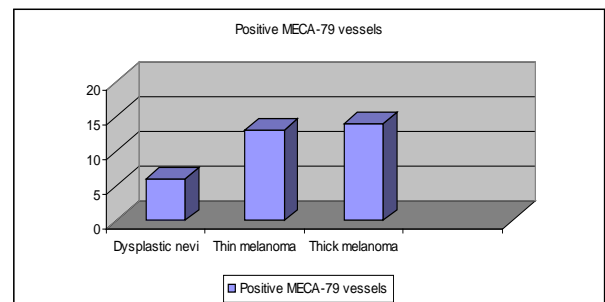


Fig.6. Situation of MECA-79 positive vessels in the pigmented lesions included in the study

In a similar study, Martinet et al.(8) detected a high number of HEVs in Dubreuilh melanoma followed by superficial spreading melanoma.

No significant correlation was found between MECA-79+vessels and sex, gender, ulceration, Breslow thickness, Clark level.

Presence of high endothelial vessels can predict lymphocytes infiltration by governing lymphocyte recruitment and may represent a local vascular respons facilitating the migration of lymphocytes into tumor tissue. [9,10]

Conclusions

A better understanding of tumor microenvironment and mechanisms involved in anti-tumor response can play an important role in development of future melanoma therapeutic strategies.

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