ORIGINAL PAPER

# TUMOR-INFILTRATING CD1A+ LANGERHANS CELLS IN PRIMARY CUTANEOUS MELANOMA – AN IMMUNOHISTOCHEMICAL STUDY

Mihaela Antohe[1,4], Ionela Hulea[1], Anastasia Coman[1,4], Alice Brînzea[1,4], Roxana Ioana Nedelcu[1,4], Gabriela Turcu[1,3,4], Andreea Moroianu[4], Mihaela Balaban[4], Lorena Manea[1], David Niculescu Mihaela Cristina[5], Elena Bălășescu[1], Sabina Zurac[1,2], Mirela Cioplea[2], Cristiana Popp[2], Luciana Nichita[2], Daniela Adriana Ion[1]

[1]"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[2]Colentina Clinical Hospital, Pathology Department, Bucharest, Romania
[3]Colentina Clinical Hospital, Dermatology Department, Bucharest, Romania
[4]Derma360 Clinic, Dermatology Department, Bucharest, Romania
[5] Dr. Leventer Centre Clinic, Dermatology Department, Bucharest, Romania

Corresponding author: Ionela Hulea
Email: dr.ionelahulea@yahoo.com

**ABSTRACT**

*We highlight in this paper the main immunological roles of dendritic cells (DCs) in cancer, specifically in melanoma. The presence of dendritic cells in the tumor microenvironment triggers an effective antitumor immunity by recruiting of infiltrating tumor lymphocytes. Moreover, DCs also have the role of regulating and maintaining immune responses. DCs are present in both normal skin and melanocytic skin lesions. At the normal skin level, DCs are predominantly in the epidermis with few located in the dermis and few others that migrate in the circulation. However, in melanocytic lesions such as benign and dysplastic nevi, an increase in the density of DCs in the dermis is observed, with coexpression of CD1 and S100 markers. The aim of the study is to investigate CD1a expression in primary cutaneous melanoma. Regarding this, we conducted a retrospective transverse study of 37 cases of primary superficial spreading melanoma. We analyzed the clinical and histopathological aspects of our group of study and we performed some correlations between CD1a expression and clinical and histopathological parameters. These correlations were tested using nonparametric tests such as Pearson and Spearman tests. The threshold for p values was considered statistically significant for 0.05. After performing Pearson test we found that CD1a positivity correlates statistically with Breslow index (p=0.017, r=0.434), Clark level (p=0.012, r=0.454), mitotic rate (p=0,036, r=0,390) and the ulceration of the tumor (p=0.044, r=0.370), with a moderate statistical power. Spearman test also showed positive correlation between CD1a expression and Breslow thickness (p=0,001, Sperman's rho=0,593), Clark level (p=0,10, Sperman's rho=0,465) and tumor ulceration (p=0,033, Sperman's rho=0,391).*

**KEYWORDS**: *CD1a expression, dendritic cells, Langerhans cells, melanoma, immunohistochemical analysis*

## INTRODUCTION

Dendritic cells (DCs), a specialized type of leukocytes, play an important role in tumor immunotherapy [1]. Considering the latest findings related to the biology of these cells, we emphasize in this paper the main immunological roles of DCs in cancer, especially in melanoma. It has been found that the presence of dendritic cells in the tumor produces the recruitment of infiltrating tumor lymphocytes, thus leading to the triggering of an effective antitumor immunity. It seems that the density and distribution of DCs at the tumor level influence the activation of T lymphocytes [2]. The study of tumor-associated dendritic cell (TADC) density and distribution is most commonly done by histological and immunohistochemical evaluation using CD1a and S-100 antigens as markers [3]. Numerous studies have shown that the prognosis of cancer patients depends on the degree of tumor infiltration by DCs [3] – [5]. Research revealed that elevated TADC levels are associated with better prognosis in gastric [6], esophageal [7], nasopharyngeal carcinomas [8] and colorectal adenocarcinoma [9] and also with longer survival and lower risk of metastasis [4]. It has been observed that some tumors are able to inhibit the production, function and survival of DCs, thus leading to an inefficient antitumor immune response and rapid tumor progression [3], [10], [11].

DCs were first described in 1973 by Ralph Steinman and as all antigen presenting cells (APCs) play an essential role in the innate and acquired immune responses [12]. They are the most important APCs and have special immunological functions in initiating, regulating and maintaining immune responses. They identify pathogenic antigens using specific receptors, process them through phagocytosis and micropinocytosis into small peptides, create specific MHC-peptides complexes and present them to CD4+ and CD8+ cells [1]. All these mechanisms initiate the host's immune responses with the activation of helper T-cells and killer T-cells as well as B lymphocytes activation [2], [13] – [15].

Immunohistochemical studies have shown that DCs most frequently express CD1 and S-100 markers on their surfaces. There are three types of CD1 markers, namely CD1a, CD1b and CD1c. Each of these markers can be found expressed as follows: at the level of cutaneous dendritic cells (Langerhans cells – LCs), CD1a and CD1c are frequently expressed and at the dermal level and at the level of migratory LCs, CD1c is mostly expressed. In addition, it has been observed that the DCs located in skin-draining lymph nodes express CD1a, CD1b and also CD1c [2], [16].

DCs are expressed in both normal skin and melanocytic skin lesions. At the normal skin level, DCs are predominantly arranged in the epidermis with few located in the dermis and few others that migrate in the circulation. However, in melanocytic lesions such as benign and dysplastic nevi, an increase in the density of DCs in the dermis is observed, with coexpression of CD1 and S100 markers [17]. In melanomas, it has been observed a decrease in the density of CD1+ DCs in the epidermis and a marked decrease in thick tumors. However, the density of DCs in the dermis remains similar to normal skin, thus excluding the hypothesis that in melanoma, dendritic cells migrate from epidermis to dermal level [18], [19]. In melanoma, DCs from epidermis are mostly CD1a+ LCs [2].

## MATERIALS AND METHOD

The aim of the study is to investigate CD1a expression in primary cutaneous melanoma, knowing that it represents a key factor in cancer progression and immunotherapy. We conducted a retrospective transverse study of 37 cases of primary superficial spreading melanoma previously diagnosed in the Pathology Department of Colentina Clinical Hospital during 2012-2016, using conventional histopathological techniques. All patients included in the study had an informed consent signed and the study was approved by the Ethics Committee of Colentina Clinical Hospital. For this study we highlighted CD1a markers by immunohistochemical staining on formalin-fixed, paraffin-embedded blocks of primary melanoma tumors. The slides were subsequently analyzed using a scale of intensity of CD1a cell positivity, as follows: 0 for the absence of positive cells, 1 for rare positive cells and 2 for frequent positive cells.

All data were collected in a database and statistically analyzed using Microsoft Excel and

IBM SPSS Statistic Data Editor v26.0. We analyzed the clinical and histologic aspects of our group of study and we made some correlations between CD1a expression and clinical and histopathological parameters. These correlations were tested using nonparametric tests such as Pearson and Spearman tests and we also evaluated the p-value and r-square in order to check the statistical significance of the results obtained. The threshold for p values was considered statistically significant for 0.05.

## RESULTS

The sample consisted of 19 (51.4%) female patients and 18 (48.6%) male patients with the mean age at the time of diagnosis of 58 years (SD = 15.8). The minimum age at the time of diagnosis was 33 years and the maximum age was 87 years. The patient distribution by age and gender is presented in Figure 1.

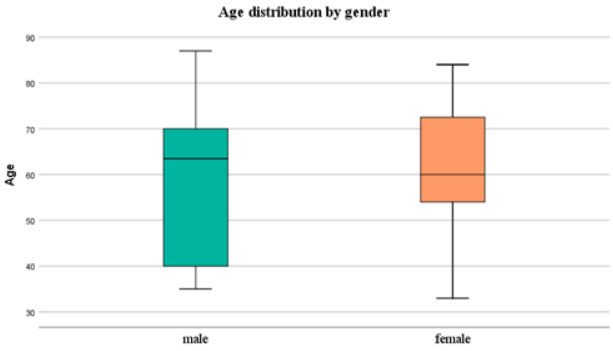


**Figure 1 – Patient distribution by gender and age in the lot of study**

The most common anatomical site of tumor occurrence was the anterior part of the trunk (35.1%). Regarding tumor thickness, according to Breslow, the mean value was 1.41 mm (SD = 1.91). In the range <0.25 mm there were 2 patients (5.4%), from 0.25 to 0.49 mm – 10 patients (27.0%), from 0.5-0.74 mm – 7 patients (18.9%), from 0.75 to 0.99 mm – 2 patients (5.4%), from 1 to 1.9 mm – 7 patients (18.9%) and > 2 mm – 9 patients (24.3%).

Level I invasion according to Clark was found in 1 patient (2.7%), level II in 14 patients (37.8%), level III in 4 patients (10.8%), level IV in 17 patients (45.9%) and level V in 1 patient (2.7%). The presence of ulceration was found in 10 patients (27%) and the existence of tumor regression was observed in 26 patients (70.3%). Regarding mitotic index, there were 29 cases (78.4%) in the range 0 to 5 mitosis, 3 cases (8.1%) from 6 to 10 mitosis and 4 cases (10.8%) with more than 11mitosis.

Lymphocytic infiltration was detected in all cases. The presence of intra-tumoral inflammatory infiltrate was highlighted in 18 of the patients (51.4%), the peri-tumoral inflammatory infiltration in 28 of the patients (80%) and the presence of both intra- and peri-tumoral infiltrate in 11 patients (31.4%).

From all the 37 cases of primary cutaneous melanoma, the CD1a expression was different (Figure 2): 16,67% had no CD1a expression (Figure 3), 50% had low positivity (Figure 4) and 33,33% of the tumors had frequent positive cells (Figure 5).

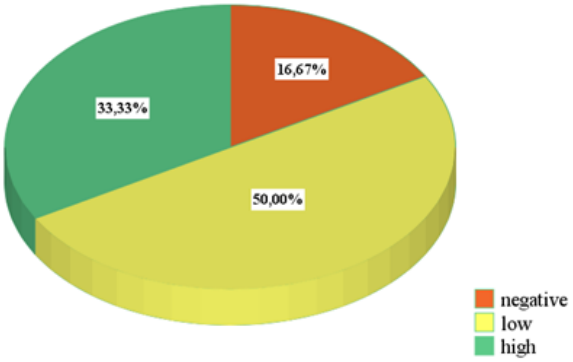


**Figure 2 – CD1a expression in the group of study**

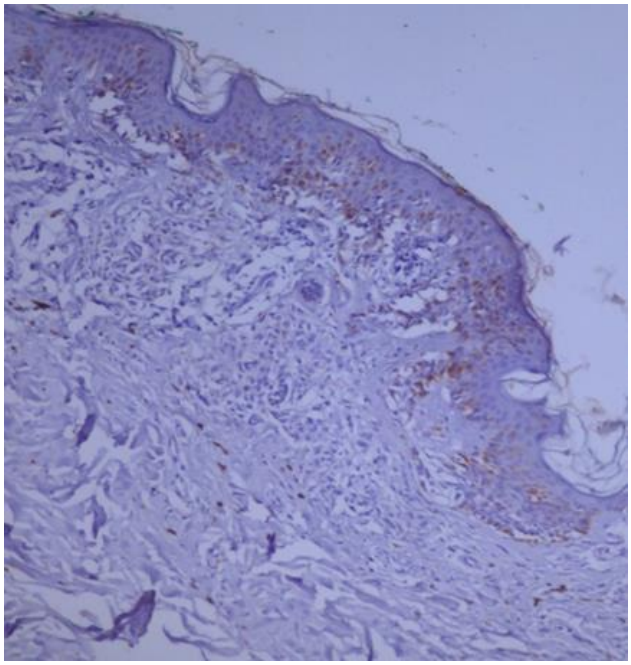

**Figure 3 – Malignant melanoma, CD1a immunostaining negative x10**

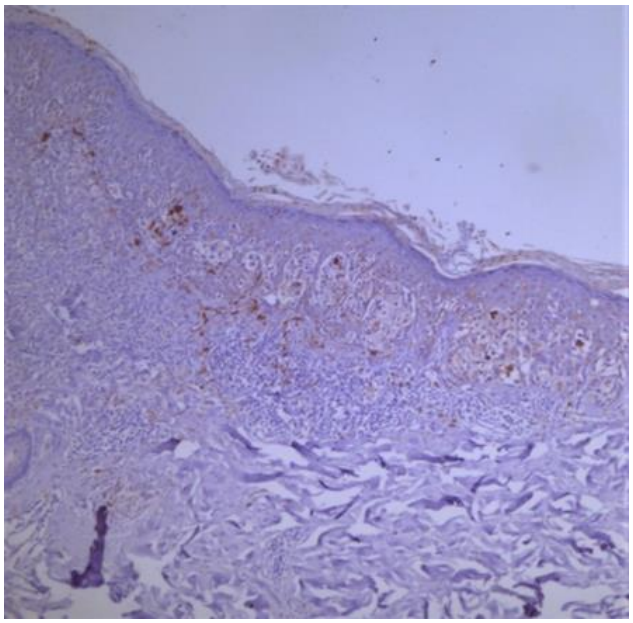

**Figure 4 – Malignant melanoma, CD1a immunostaining positive in rare cells x5**

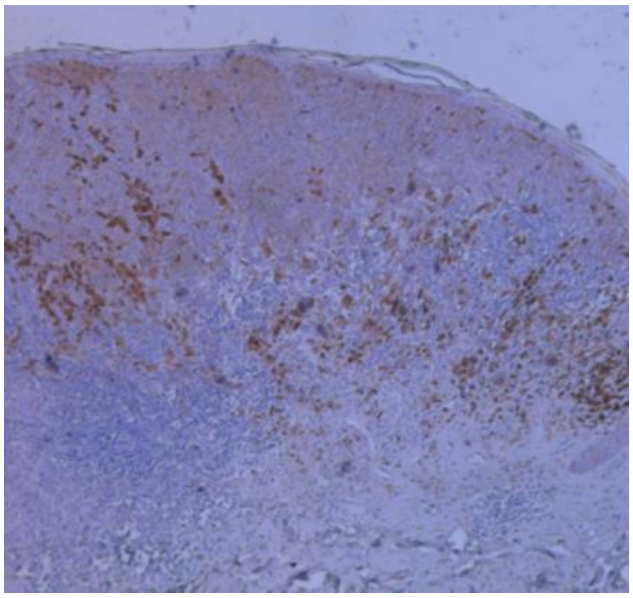

**Figure 5 – Malignant melanoma, frequent Langerhans cells positivity (CD1a immunostaining x10)**

Of the patients with rare CD1a positive cells, most had Clark level between I and III and in those melanomas with high CD1a positivity, IV and V Clark level predominated. Patient distribution by Clark index and CD1a positivity is represented in Figure 6.

Regarding melanoma cases that showed regression, 81% had CD1a positive expression. Of these, 52.2% of them had rare positive cells and 47.6% had frequent positive cells. All cases of melanoma without regression showed CD1a-positive cells, of which 54.5% with rare positive cells and 45.5% with frequent positive cells.

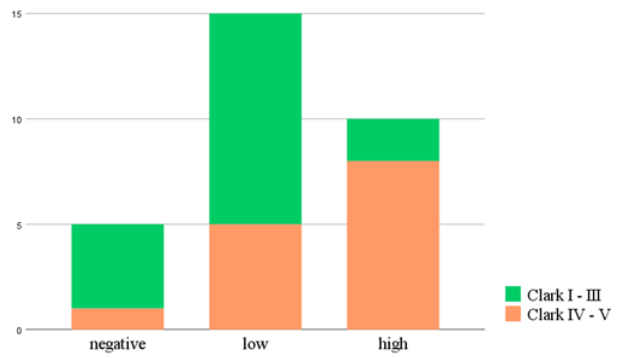


**Figure 6 – Patient distribution by Clark level and CD1a positivity**

We made Pearson and Spearman correlation between CD1a expression and clinical and histological parameters of these 37 cases of superficial spreading melanoma. After performing Pearson test we found that CD1a positivity correlates statistically with Breslow index (p=0.017, r=0.434), Clark level (p=0.012, r=0.454), mitotic rate (p=0,036, r=0,390) and the ulceration of the tumor (p=0.044, r=0.370), with a moderate statistical power. Spearman test also showed positive correlation between CD1a expression and Breslow thickness (p=0,001, Sperman's rho=0,593), Clark level (p=0,10, Sperman's rho=0,465) and tumor ulceration (p=0,033, Sperman's rho=0,391). We found no correlation between CD1a positivity and tumor regression.

Another correlation with statistical significance was between Breslow index and age (p=0,015, r=0,398), Breslow and Clark level (p=0,03, r=0,477) and Breslow and FoxP3 (p=0,004, r=0,487).

## DISCUSSION

The group of selected patients was relatively homogeneous in terms of the gender of the patients with a mean age at the time of diagnosis of 58 years, for both women and men. The anterior part of the trunk was the most common location of the tumor. The mean value of Breslow depth was 1.41 mm and the most values were between 0.25 and 0.49 mm. In terms of degree of invasion according to Clark, level IV was the most common. Lymphocytic infiltration was identified in almost all patients with intra-tumoral distribution in 51.4% of the cases and peri-tumoral distribution in 80 % of the cases.

In the present study we used CD1a immunostaining to highlight Langerhans cells due to its demonstrated specificity for these cells, especially in the epidermis [20], [21]. The expression of CD1a was statistically correlated with Breslow index, Clark level, mitotic rate and the ulceration of the tumor. Regarding patient distribution by Clark index and CD1a positivity, the most frequent CD1a positive tumor cells were identified in cases of Clark IV and V levels.

## CONCLUSION

We performed an immunohistochemical study of dendritic cells in melanoma and we observed that most of the tumors (83.3%) express CD1a positivity. We obtained statistically significant correlation between Breslow index, Clark level, mitotic rate, ulceration and CD1a expression. We propose further studies in which to introduce more melanoma patients in our study group and in addition to comparatively analyze CD1a expression in melanocytic nevi.

## REFERENCES

[1] Y. Wang et al., "Dendritic cell biology and its role in tumor immunotherapy," J. Hematol. Oncol., vol. 13, no. 1, 2020.
[2] M. R. Hussein, "Dendritic cells and melanoma tumorigenesis an insight," Cancer Biol. Ther., vol. 4, no. 5, pp. 501–505, 2005.
[3] L. Perez, M. R. Shurin, B. Collins, D. Kogan, I. L. Tourkova, and G. V. Shurin, "Comparative analysis of CD1a, S-100, CD83, and CD11c human dendritic cells in normal, premalignant, and malignant tissues," Histol. Histopathol., vol. 20, no. 4, pp. 1165–1172, 2005.
[4] Y. Becker, "Anticancer role of dendritic cells (DC) in human and experimental cancers - A review," Anticancer Research, vol. 12, no. 2. pp. 511–520, 1992.
[5] F. O. Nestle, G. Burg, J. Fäh, T. Wrone-Smith, and B. J. Nickoloff, "Human sunlight-induced basal-cell-carcinoma-associated dendritic cells are deficient in T cell co-stimulatory molecules and are impaired as antigen- presenting cells," Am. J. Pathol., vol. 150, no. 2, pp. 641–651, 1997.
[6] S. Tsujitani, Y. Kakeji, A. Watanabe, S. Kohnoe, Y. Maehara, and K. Sugimachi, "Infiltration of dendritic cells in relation to tumor invasion and lymph node metastasis in human gastric cancer," Cancer, vol. 66, no. 9, pp. 2012–2016, 1990.
[7] W. Yang and J. Yu, "Immunologic function of dendritic cells in esophageal cancer," Digestive Diseases and Sciences, vol. 53, no. 7. Springer, pp. 1739–1746, 2008.
[8] H. Nomori, S. Watanabe, T. Nakajima, Y. Shimosato, and T. Kameya, "Histiocytes in nasopharyngeal carcinoma in relation to prognosis," Cancer, vol. 57, no. 1, pp. 100–105, 1986.
[9] K. Ambe, M. Mori, and M. Enjoji, "S-100 protein-positive dendritic cells in colorectal adenocarcinomas. Distribution and relation to the clinical prognosis," Cancer, vol. 63, no. 3, pp. 496–503, 1989.
[10] N. S. Katsenelson, G. V Shurin, S. N. Bykovskaia, J. Shogan, and M. R. Shurin, "Human small cell lung carcinoma and carcinoid tumor regulate dendritic cell maturation and function," Mod. Pathol., vol. 14, no. 1, pp. 40–45, 2001.
[11] T. Oyama et al., "Vascular endothelial growth factor affects dendritic cell maturation through the inhibition of nuclear factor-kappa B activation in hemopoietic progenitor cells.," J. Immunol., vol. 160, no. 3, pp. 1224–1232, 1998.
[12] P. Fang et al., "Immune cell subset differentiation and tissue inflammation," Journal of Hematology and Oncology, vol. 11, no. 1. BioMed Central Ltd., 2018.
[13] M. B. Lutz and G. Schuler, "Immature, semi-mature and fully mature dendritic cells: Which signals induce tolerance or immunity?" Trends in Immunology, vol. 23, no. 9. Trends Immunol, pp. 445–449, 2002.
[14] Z. Chen, X. Zhang, J. Xiang, and J. R. Gordon, "Analysis of the gene expression profiles of immature versus mature bone marrow-derived dendritic cells using DNA arrays," Biochem. Biophys. Res. Commun., vol. 290, no. 1, pp. 66–72, 2002.
[15] M. C. Dieu et al., "Selective recruitment of immature and mature dendritic cells by distinct chemokines expressed in different anatomic sites," J. Exp. Med., vol. 188, no. 2, pp. 373–386, 1998.
[16] E. Jörundsson, C. M. L. Press, and T. Landsverk, "Distribution of MHC-II and CD1 molecules in the skin of lambs and changes during experimentally-induced contact hypersensitivity," Vet. Immunol. Immunopathol., vol. 74, no. 1–2, pp. 87–101, 2000.
[17] E. B. Bröcker, C. Reckenfeld, H. Hamm, D. J. Ruiter, and C. Sorg, "Macrophages in melanocytic naevi," Arch. Dermatol. Res., vol. 284, no. 3, pp. 127–131, 1992.
[18] D. Garcia-Plata et al., "Immunophenotype analysis of dendritic cells and lymphocytes associated with cutaneous malignant melanomas," Invasion and Metastasis, vol. 15, no. 3–4, pp. 125–134, 1995.
[19] K. Toriyama, K. D.-R. Wen, E. Paul, and A. J. Cochran, "Variations in the Distribution, Frequency, and Phenotype of Langerhans Cells

During the Evolution of Malignant Melanoma of the Skin.," J. Invest. Dermatol., vol. 100, no. 3, pp. 269S-273S, 1993.

[20] N. Mizumoto and A. Takashima, "CD1a and langerin: acting as more than Langerhans cell markers," J. Clin. Invest., vol. 113, no. 5, pp. 658–660, 2004.

[21] S. W. Kashem, M. Haniffa, and D. H. Kaplan, "Antigen-presenting cells in the skin," Annual Review of Immunology, vol. 35. Annual Reviews Inc., pp. 469–499, 2017.