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Dendritic cells in melanoma – immunohistochemical study and research trends

ROXANA-IOANA NEDELCU[1)], DANIELA-ADRIANA ION[1)], COSMIN ADRIAN HOLEAB[2)], MIRELA-DANIELA CIOPLEA[3)], ALICE BRÎNZEA[1)], SABINA ANDRADA ZURAC[3)]

[1)]*Department of Pathophysiology II, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania*

[2)]*United Nations Educational, Scientific and Cultural Organization (UNESCO) Chair on Science and Innovation Policies, National University of Political Studies and Public Administration (SNSPA), Bucharest, Romania*

[3)]*Department of Pathology, Colentina Clinical Hospital, Bucharest, Romania; "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania*

Abstract

Cutaneous dendritic cells play multiple physiological roles and are involved in various pathophysiological processes. Research studies of dendritic cells abound in the medical literature. Nevertheless, the role of dendritic cells in melanoma regression phenomenon is not completely understood. We conducted a scientometric analysis in order to highlight the current state on research regarding dendritic cells and melanoma. We also performed an immunohistochemical study, using specific markers for dendritic cells (CD1a, langerin). We evaluated the frequency and distribution of dendritic cells in areas of tumor regression compared to the areas of inflammatory infiltrate of melanoma without regression. The immunohistochemical study we performed revealed that dendritic cells are more frequent in the regressed areas, comparing with non-regressed ones. In regressed areas, dendritic cells have a predominant nodular pattern (19 cases), followed by diffuse isolate pattern (eight cases) and mixed pattern (diffuse and nodular) (three cases). In melanoma without regression, most cases presented a diffuse pattern (27 cases) of dendritic cells distribution. In conclusion, our immunohistochemical study stressed differences between frequency and distribution of dendritic cells located in the melanoma with regression and melanoma without regression. These data suggest that dendritic cells are involved in the regression phenomenon. Following the literature analysis we obtained, we observed that dendritic cells profile in melanoma with regression was poorly studied. Insights into antitumor immune response and dendritic cells may be essential for the understanding of the potential prognostic role of dendritic cells in melanoma and for the development of new promising therapeutic strategies for melanoma.

Keywords: melanoma/skin cancer, dendritic cells, tumor regression, Langerhans cells, cancer vaccine, immunotherapy.

Introduction

Dendritic cells (DCs) normally found in the skin are Langerhans cells (in the epidermis) and dermal dendritic cells [1]. In inflammatory disorders, different subtypes of DCs have also been identified: inflammatory dendritic cells and plasmacytoid dendritic cells. Due to the many roles of dendritic cells in skin physiology and in skin diseases pathophysiology [2], medical literature abounds in diverse information regarding cutaneous dendritic cells. Therefore, we considered useful to perform an analysis focusing on the relation between dendritic cells and melanoma.

The role of dendritic cells is complex, DCs representing a modulator of skin immunity, involved in both adequate immunological reactions and immune tolerance [3]. Extremely important in antitumor immune defenses is the capacity of Langerhans cells to present antigens to CD8+ T-lymphocytes *via* histocompatibility complex class I [4, 5]. Targeting tumor antigens by Langerhans cells could be an effective strategy in antitumor therapy. This hypothesis led to the development of new therapeutic concepts for topical immunization by directly introducing the Langerhans cells in the skin with antigens [6]. Epicutaneous immunization with antigens in cutaneous lesions determined potent cytotoxic response of CD8+ T-lymphocytes, in order to inhibit growth of melanoma [7]. An essential phenomenon, in the evolution of melanoma, is the spontaneous tumor regression. Regression of melanoma is the result of a complex interaction between malignant melanocytes and the host immune response [8].

Being an immunogenic skin cancer, melanoma may benefit from active immunotherapy. Dendritic cells, professional antigen-presenting cells with a crucial role for the induction of antitumor responses, were considered suitable immune adjuvants for vaccine therapy. Despite the attractive properties of DCs, it has yet not been identified ideal DCs source, delivery system, dosing schedule for therapeutic DC vaccine. Future studies are needed to obtain an optimal adjuvant role of DCs in melanoma patients' management.

Aim

The aim of the study was to analyze the frequency and the distribution of dendritic cells in melanoma with regression and melanoma without regression. We also intended to highlight the current state on research regarding dendritic cells and melanoma.

Materials and Methods

Literature analysis

A *Scopus* search in February 2015, using keywords

"dendritic cell" and "melanoma", limited to the period 2012–2015 and the English language, was performed and resulted 712 articles. We created a file containing key words indicated by the authors of articles and a folder containing the full text articles, both associated with the year of publication of articles. The semantic study involved several steps. In first stage, we removed from the full text articles the unnecessary text (abstracts, footnotes, references, acknowledgements) that can bias semantic and statistical results.

In the second stage, we created a semantic dictionary, which contains 54 semantic references and hundreds of keywords using Tropes software. Semantic dictionary processing methods were conducted so that the dictionary would reflect the semantic content of the 712 analyzed articles and be relevant for the scientometric study. Finally, we draw the graphs below, which are visual representations of semantic relations between terms. These statistical results were achieved through network analysis Gephi software. The input used in Gephi is the ontology of terms, defined and exported from Tropes. The thickness of the connection line indicates the intensity of semantic relations, the size of the nodes indicates the frequency in text.

Histology and immunohistochemistry

We selected 60 consecutive cases of patients with clinical and histopathological diagnosis of melanoma. Selected patients showed similarities of age, gender and anatomical location of the tumors. Thirty of 60 cases of melanoma presented partial or segmental tumor regression, the remaining 30 cases being melanoma without regression areas. We obtained ethical approval and consent of patients for the study.

The skin tumor samples were received in the Laboratory of Pathology, in special containers, in 10% formalin. The samples were fixed for 24 hours, then processed using tissue processor Thermo Fisher Microm STP 420D and included in paraffin. We performed 3 μm thick sections using a semi-automatic Leica RM2245 rotary microtome; sections were placed on precoated slides for immunohistochemical tests.

Immunohistochemical (IHC) tests were performed for the following antigens: CD1a and langerin (Table 1). As detection system, we used Polymer Novolink (Leica/ Novocastra). Immunohistochemical staining was analyzed using a microscope Nikon 80i.

Table 1 – ***Immunohistochemical tests***

Primary antibody	Clone	Host	Source	Dilution	Specificity
CD1a	MTB1 mono-clonal	Mouse	Leica Biosystems	1:50	Human CD1a molecule
Langerin	12D6 mono-clonal	Mouse	Leica Biosystems	1:50	Human langerin

After performing immunohistochemical tests, we evaluated the frequency and distribution of dendritic cells in areas of tumor regression compared to the areas of inflammatory infiltrate of melanoma without regression.

Results

The scientometric analysis we performed revealed the actual scientific trends regarding dendritic cells and melanoma. Due to the ability to induce anti-tumor reactions, DCs represents a promising therapeutic option in melanoma. It is important to enrich the knowledge regarding ontogeny, diversity and properties of skin dendritic cells [9]. Because of ethical principles on human research, melanoma animal models had a key part in scientific progress regarding DCs and melanoma [10].

Our analysis highlighted the importance of semantic relation between "animal model" and "cancer vaccine" found in medical literature in recent years (Figure 1, A and B).

Thus, in the attempt to elucidate immunological mechanisms of melanoma, different animal models of melanoma were created: small rodents, guinea pigs, opossum, and fishes. Using mouse melanoma models, progress has been made in understanding the molecular mechanisms. However, many questions about the melanoma infiltrating DCs are still unanswered [10]. In a recent published study, the authors reported interesting results using a vaccine with *Listeria monocytogenes* to treat an experimental melanoma model in mice: it was described the transformation of melanocytes into dendritic cells and the regression of melanoma; the vaccine was also safe, the use of antibiotic therapy was not necessary in order to eliminate the bacteria [11].

One of the biggest challenges of specialists from different clinical and fundamental research disciplines is to increase immune system capacity so that it can effectively mediate the complete regression of cancer. Immunological therapy purposes in melanoma aim to boost the immune system to recognize, to fight, to destroy and to remember cancer cells. Vaccine may be an effective therapeutic option in melanoma, this method being an important topic for specialized medical literature, as we stressed in Figure 1C.

Due to the essential role that DCs play in the immune system, of potent antigen presenting cells and potent initiators of immune responses, DCs are commonly used in cancer vaccines. Vaccine therapy in melanoma was well tolerated and it has the advantage of being used in combination with other treatments. Despite antitumoral properties of DCs, results of clinical trials have been disappointing.

We proposed to study the frequency and distribution of dendritic cells in melanoma with regression and melanoma without regression. We analyzed the results of IHC tests for Langerhans cells (CD1a and langerin). We identified different patterns of expression depending on the area examined (Figures 2 and 3).

Dendritic cells are more frequent in the regressed areas, comparing with non-regressed ones. In regressed areas, dendritic cells have a predominant nodular pattern (19 cases), followed by diffuse isolate pattern (eight cases) and with mixed pattern (diffuse and nodular) (three cases). In melanoma without regression, most cases presented a diffuse pattern (27 cases) of dendritic cells distribution (Figure 4).

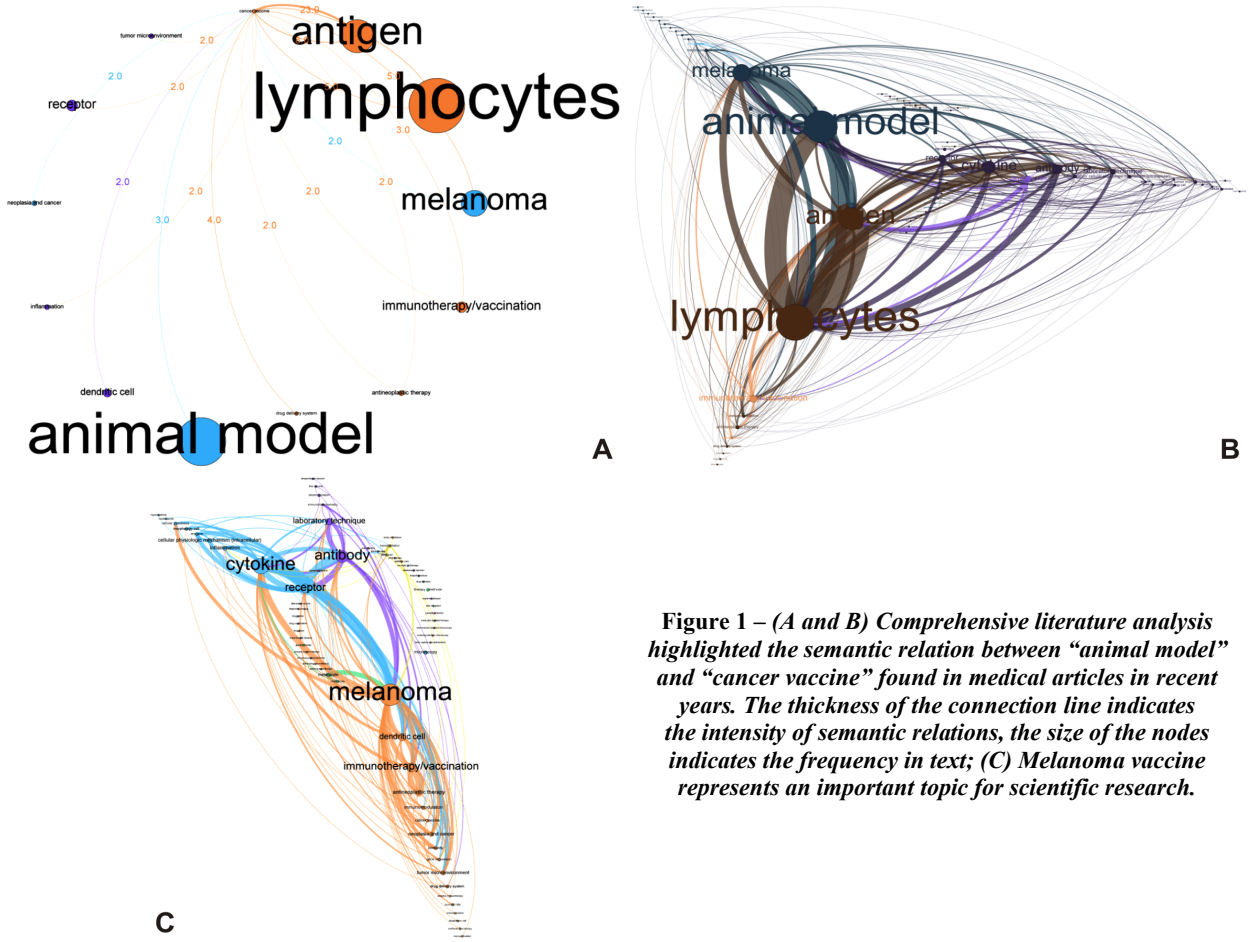

Figure 1 – ***(A and B) Comprehensive literature analysis highlighted the semantic relation between "animal model" and "cancer vaccine" found in medical articles in recent years. The thickness of the connection line indicates the intensity of semantic relations, the size of the nodes indicates the frequency in text; (C) Melanoma vaccine represents an important topic for scientific research.***

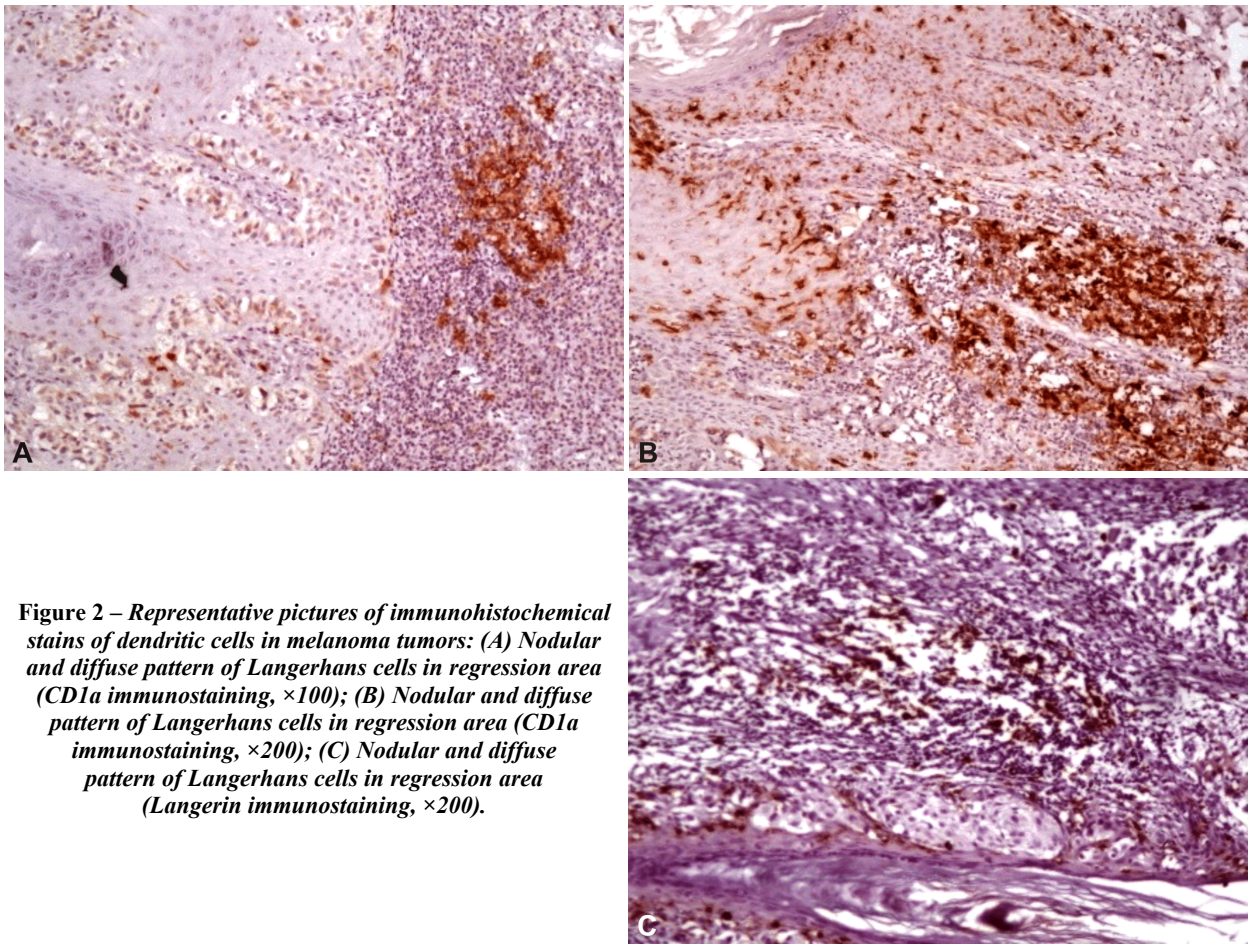

Figure 2 – ***Representative pictures of immunohistochemical stains of dendritic cells in melanoma tumors: (A) Nodular and diffuse pattern of Langerhans cells in regression area (CD1a immunostaining, ×100); (B) Nodular and diffuse pattern of Langerhans cells in regression area (CD1a immunostaining, ×200); (C) Nodular and diffuse pattern of Langerhans cells in regression area (Langerin immunostaining, ×200).***

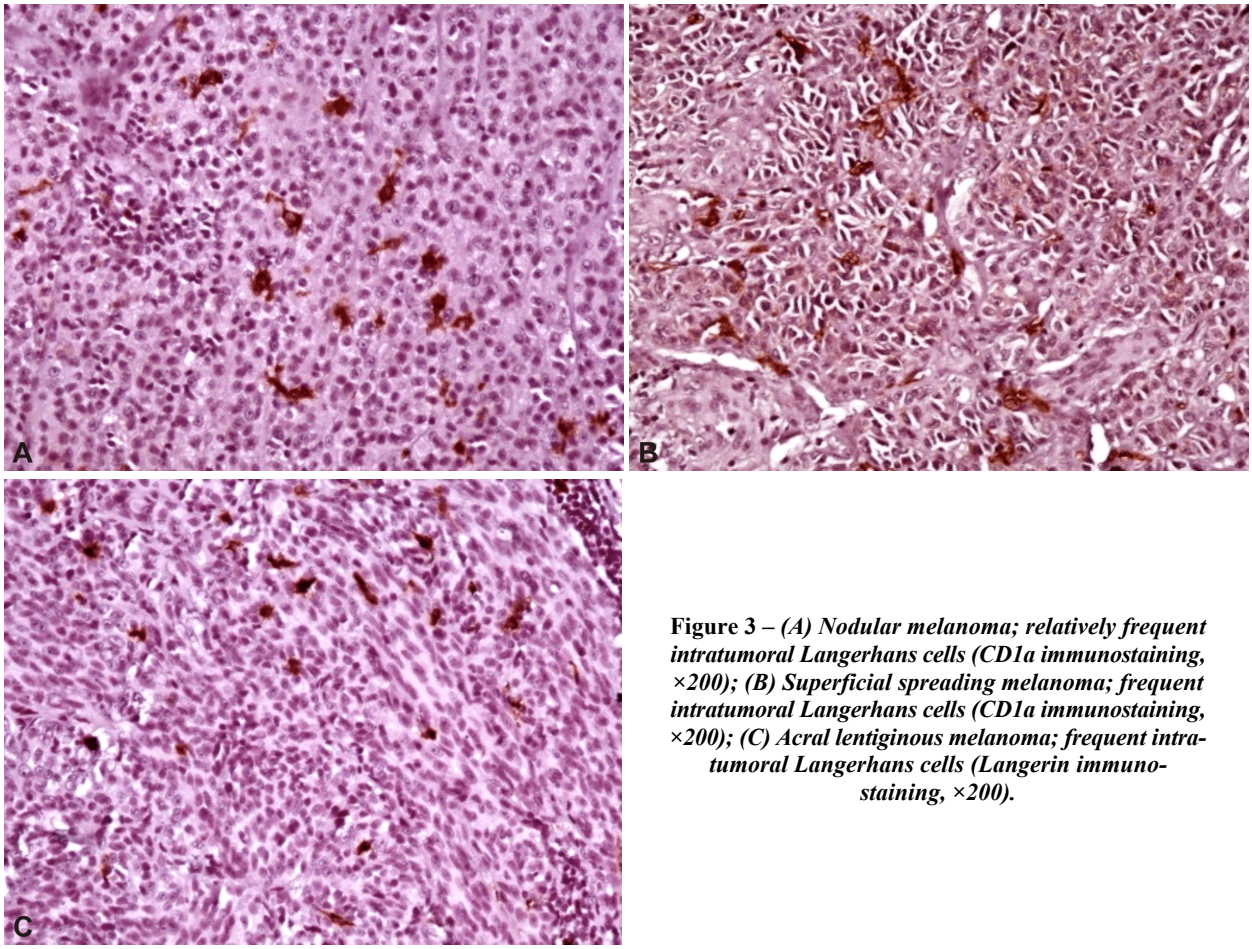

Figure 3 – ***(A) Nodular melanoma; relatively frequent intratumoral Langerhans cells (CD1a immunostaining, ×200); (B) Superficial spreading melanoma; frequent intratumoral Langerhans cells (CD1a immunostaining, ×200); (C) Acral lentiginous melanoma; frequent intratumoral Langerhans cells (Langerin immunostaining, ×200).***

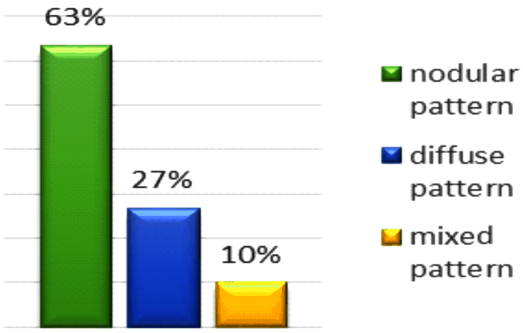

Figure 4 – ***Distribution of dendritic cells in melanoma with regression.***

Discussion

Dendritic cells (DCs) present in the inflammatory infiltrate of melanoma can have different roles. Dendritic cells identified in melanoma may induce both immune tolerance towards the tumor [12] and anti-tumor immune responses [13]. Inefficient immune responses induced by dendritic cells in some cases of melanoma can be explained by DC dysfunction induced by melanoma. In the absence of the molecules produced by the tumor, differentiation and maturation of DCs are adequately. Matured DCs have increased capacity to process antigens and to express cytokines and co-stimulatory molecules, essential for initiating an effective immune response [14]. Under the influence of tumoral factors, apoptotic and immature DCs are generated and accumulated. Although immature DCs do not have the capacity to present antigens, it can suppress antitumor T-cell activity through the production of oxygen free radicals. DCs with antigen presenting capacity, in the absence of appropriate co-stimulatory molecules and cytokine secretion, can generate reactions that favor the tumoral expansion [12].

Thus, the optimization of anti-melanoma vaccine represents an important objective for dermatologists, oncologists, immunologists, pathologists, clinical and laboratory researchers. One field in which we need new results is represented by the insights into dendritic cells used for anti-melanoma vaccine. The optimal type of DCs to be used, the stage of maturation of DCs, the quantity used, the route and frequency of administration are some of the topics of current research. On the other hand, insufficient knowledge of local immunopathology prevented development of an ideal immunotherapy. New studies will contribute to the development of methods to counter the peritumoral immunosuppressive environment that induces host tolerance to cancer. Stimulating factors were used for activating DCs and obtaining mature DCs both *in vitro* and *in vivo*. Inflammatory cytokines and Toll-like receptors are used in the *in vitro* cultures for many years [15, 16]. Besides concern to elicit an effective anti-cancer response of specific lymphocytes, progress was made on the induction of immunological memory, control of tumor recurrence and metastasis. In a recent study that used TriMixDC-MEL, a vaccine in which DCs were electroporated with mRNA encoding CD40 ligand (CD40L), CD70 and a constitutive active form of TLR4

(caTLR4), a durable antitumoral activity was obtained in a subset of patients with melanoma [17].

Following the analysis obtained, we observed that dendritic cells profile in melanoma with regression was poorly studied. The prognostic value of regression in melanoma is not completely understood. The subject of regression is still controversial, both because of discordant results and the fact that many observations are due to reports of sporadic cases, because of insufficient large studies. Initially, it was considered that anti-tumor reactions against the malignant melanocytes may be suggestive of favorable disease evolution. On the other hand, the resorption of melanoma is only partial in most cases, hence malignant clones can survive undamaged, not being recognized by the immune system. Some authors have suggested that melanoma regression may be a negative prognosis factor [18].

In a recent study of immunophenotypic inflammatory cells in melanoma with regression and halo nevus, the authors emphasize the need of immunohistochemical study of dendritic cells in melanocytic tumors with regression [19]. In another study, we observed that the presence of Langerhans cells in melanoma tumor mass was associated with: tumor type, Langerhans cells being better represented in superficial spreading melanoma when compare with nodular melanoma and acral lentiginous melanoma; Breslow index, Langerhans cells becoming less represented as the tumor pT stage increases [20]. A recent study about immunomics in skin cancers also highlighted the importance of identifying predictive biomarkers in order to personalize treatments in melanoma [21].

Significant increase of the number of dendritic cells, as well as their particular distribution in areas of regression can indicates that they are involved in regression mechanisms and have potential prognostic value for melanoma patients.

Conclusions

We performed an immunohistochemical study of dendritic cells (DCs) in melanoma. We analyzed the DC's frequency and pattern of distribution in melanoma with and without regression. We observed differences regarding predominant patterns of DCs in areas of tumor regression compared to the areas of inflammatory infiltrate of melanoma without regression. In regressed areas, DCs have a predominant nodular pattern (19 cases); in melanoma without regression, most cases presented a diffuse pattern (27 cases) of DCs distribution. The particular distribution of DCs in melanoma with regression and the increased number of DCs in areas of regression comparing with non-regressed areas can indicate that DCs are involved in regression phenomenon and may have prognostic value in melanoma. Following the literature analysis, we performed, we observed that dendritic cells profile in melanoma with regression was poorly studied. Characterization of DCs and understanding its behavior will crucially contribute to vaccine development and management of skin diseases.

Conflict of interests

The authors declare that they have no conflict of interests.

Author contribution

All the authors had equal contributions to the article.

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Corresponding author
Roxana-Ioana Nedelcu, MD, PhD, Department of Pathophysiology II, Clinical Department No. 2, "Carol Davila" University of Medicine and Pharmacy, Bucharest; "Matei Balş" National Institute of Infectious Diseases – HIV/AIDS and Infectious Diseases Academy, 3rd Floor, Room 302, 1 Dr. Calistrat Grozovici Street, 021105 Bucharest, Romania; Phone +40721–916 624, e-mail: roxanaioana.nedelcu@yahoo.com

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