ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

Peritumoral inflammatory reaction in non-melanoma skin cancers – histological and immunohistochemical study

IOAN AVRĂMOIU[1)], ILEANA OCTAVIA PETRESCU[2)], MARIUS EUGEN CIUREA[3)], ADRIANA BOLD[4)], ISABELA SILOŞI[5)], MARILENA MONICA ŢÂNŢU[6)], MIHAELA NICULESCU[7)], RUMI EUGEN ANGHEL SAVCIU[8)], STELIAN ŞTEFĂNIŢĂ MOGOANTĂ[9)]

[1)]*PhD Student, Department of Histology, University of Medicine and Pharmacy of Craiova, Romania*
[2)]*Department of Pediatrics, Faculty of Medicine, University of Medicine and Pharmacy of Craiova, Romania*
[3)]*Department of Plastic Surgery and Reconstructive Microsurgery, University of Medicine and Pharmacy of Craiova, Romania*
[4)]*Department of Histology, University of Medicine and Pharmacy of Craiova, Romania*
[5)]*Department of Immunology, University of Medicine and Pharmacy of Craiova, Romania*
[6)]*Department of Nursing and Kinetotherapy, Faculty of Sciences, University of Pitesti, Romania*
[7)]*Department of Anatomy, University of Medicine and Pharmacy of Craiova, Romania*
[8)]*Department of Plastic Surgery and Reconstructive Microsurgery, Emergency County Hospital, Craiova, Romania*
[9)]*Department of Surgery, Faculty of Dentistry, University of Medicine and Pharmacy of Craiova, Romania*

Abstract

Non-melanoma skin cancers (NMSCs) are the most frequent types of cancer in white skin populations, all over the world. In the last 40 years, there was observed a rapid increase of their incidence, because of the UV radiations exposure and weather changes. Although its morbidity is a relatively modest one, the direct social costs of NMSCs are quite substantial due to a high incidence. Due to these reasons, numerous studies try to clarify the etiopathogenic mechanisms of NMSCs, to elaborate treatment and prevention measures. In the last years, a special attention was given to the relation between inflammation and skin cancer. In our study, we performed a histological and immunohistological evaluation of the inflammatory reaction on a number of 73 surgical exeresis pieces coming from the patients diagnosed with NMSCs. Of these, 21 were squamous cell carcinomas (SCCs) and 52 basal cell carcinomas (BCCs). The peritumoral inflammatory reaction in NMSCs was an extremely variable one in intensity and distribution, from one case to another and even from one area to another within the same tumor, thus proving the complexity of the relations between tumor cells and the cells of the immune system. By comparing the intensity of the inflammatory reaction between the two main types of NMSCs, there was observed that in SCCs the inflammatory reaction was more intense in comparison to BCCs. Also, in SCC there was highlighted a more abundant inflammatory infiltrate in poorly differentiated carcinomas, in comparison to the well-differentiated ones. The presence of the immune system cells (T-lymphocytes, macrophages, mast cells) among the tumoral cells, in a direct contact with these, makes us believe that between the two categories of cells there may appear mechanisms of intercellular communication, distinct from the mechanisms of paracrine signaling.

Keywords: non-melanoma skin cancer, chronic inflammation, ultraviolet radiation, immune cells.

Introduction

Non-melanoma skin cancers (NMSCs), represented mainly by the basal cell carcinomas (BCCs) and squamous cell carcinomas (SCCs), are a major concern of healthcare systems all over the world, due to the fact that in the last decades there was observed a rapid increase of their incidence, reaching the proportions of an epidemics [1, 2]. According to some opinions, NMSCs are the most frequent cancers all over the world [2–4]. In Australia, NMSCs include 75% of all cancer cases, and in the United States, between 1992 and 2006, the number of NMSCs increased by 76.9%, from 1 158 298 in 1992, to 2 048 517 in 2006 [5]. Also, in the United States, NMSCs represent 40% of all cancer cases [6]. Estimations of the *American Society for Cancer* suggest that every year, in the USA, there are diagnosed more than two million cases of NMSCs [7–9]. The annual costs for the care of patients with NMSCs raise up to approx. 511 million dollars in Australia [4, 10] and to about 650 million dollars in the USA [4, 11].

The most known risk factors involved in the etiopathogeny of NMSCs are represented by the UV radiations, alongside the medical irradiation with X-rays or γ rays, the presence of some chemical cancer agents in the environment or alimentation, some treatments with immunosuppressors, etc. There is estimated that UV exposure causes molecular and genetic lesions in over 90% of the NMSCs cases [12, 13]. These alarming statistics underline the importance of studying the cellular and molecular mechanisms that are at the basis of NMSCs onset, with the purpose of elaborating some prevention methods and finding new treatment strategies.

The relation between inflammation and cancer is more and more studied in various types of human tumors. Most studies suggest that acute inflammation is not associated with the onset of tumoral lesions, while chronic inflammation is frequently incriminated in carcinogenesis [14, 15].

In the present study, our purpose was to evaluate some pathological and immunohistochemical quality aspects of

the peritumoral immunohistochemical reaction in the two major types of NMSCs.

Materials and Methods

The study included a number of 73 cases of non-melanoma skin cancers admitted and operated between 2014 and 2015 in the Clinics of Surgery, Dermatology and Plastic and Reconstructive Surgery within the Clinical Emergency Hospital of Craiova, Romania. Of these, 21 cases were squamous cell carcinomas (SCCs) and 52 basal cell carcinomas (BCCs). The most frequent localization of neoplastic lesions was on the face (61 cases), on the hands (nine cases) and on the lower limbs (three cases).

After excision, the fragments of skin tumors were fixed in 10% neutral formalin solution and processed by the histological technique of paraffin inclusion. For the pathological study, there were used two stainings: Hematoxylin–Eosin (HE) staining and green light trichrome staining, the Goldner–Szekely (GS) technique. For the immunohistochemical study of the biological material included in paraffin, there were performed sections of 3-μm thickness, harvested on port-object blades, covered with poly-L-lysine (Sigma), in order to increase the section adherence to the blades, followed by the classical immunohistochemical protocol, consisting in the deparaffinization and hydration of sections, antigen demasking by boiling the sections in a sodium citrate, pH 6, for 21 minutes, blocking of endogenous peroxidase by incubating the histopathological pieces in 3% oxygenated water for 30 minutes. The blocking of non-specific sites was performed by transferring the sections into a 2% skimmed milk bath for 30 minutes.

The biological material prepared was incubated with primary antibodies for 18 hours (overnight), in a refrigerator, at 4^0C, and the next day there was applied the biotinylated secondary antibody for 30 minutes at room temperature. After washing the biological material with 1% phosphate-buffered saline (PBS), there was applied Streptavidin–HRP (Horseradish peroxidase) for 30 minutes, at room temperature, followed by blade washing in 1% PBS 3×5 minutes. The signal was detected by using 3,3'-Diaminobenzidine (DAB, Dako). There followed the contrasting with Mayer's Hematoxylin, alcohol dehydration, xylene clarification and blade setting using a DPX environment (Fluka).

For the immunohistochemical study, there were used the following antibodies:

- monoclonal mouse anti-human CD3 (clone F7.2.38, Dako, 1:100 dilution);
- monoclonal mouse anti-human CD20cy (clone L26, Dako, 1:100 dilution);
- monoclonal mouse anti-human CD68 (clone KP1, Dako, 1:200 dilution);
- monoclonal mouse anti-human mast cell tryptase (clone AA1, Abcam, 1:1000 dilution).

Results

The skin is an extremely complex structure acting like a physical and immunological barrier that constantly and efficiently responds to the aggression of external pathogenic agents, toxins or some physical agents from the external environment, due to the immune system cells that reside in various tissues of the skin. The skin immune system also plays the part of discharging the old or altered cells, deteriorated molecules and non-self structures, so as tissue homeostasis could be preserved. The molecular mechanisms by which the immune system cells become tolerant towards the tumoral cells still remain unclear.

In our study, the microscopic evaluation of peritumoral inflammatory reaction in NMSCs showed that this had an aspect of chronic reaction, mainly made up of mononuclear round cells, of the lymphocyte, plasmocyte and macrophage type. The evaluation of peritumoral inflammatory reaction in BCCs and SCCs with small microscopic objectives, for the overall analysis of images, showed major changes of the pathological aspects from one case to another, and even from one area to another within the same tumor. Thus, in BCC, there were highlighted areas of intense inflammatory reaction and areas with reduced peritumoral inflammatory reaction (Figures 1 and 2), without any other particular pathological changes to justify this heterogeneous distribution of the immune system cells. In squamous cell carcinomas, the intensity of the peritumoral inflammatory reaction seemed to be correlated with the degree of tumoral differentiation. Thus, in well-differentiated SCCs, the inflammatory reaction was low or medium (Figure 3), while in poorly or moderately differentiated carcinomas, the intensity of inflammatory reaction was high (Figure 4).

By comparing the two types of carcinomas, there was observed that in SCCs the peritumoral inflammatory reaction was more intense and more extended in comparison to BCCs. Also, there was observed that, while basal cell carcinomas have the tendency to clearly differentiate from the tumoral stroma, squamous cell carcinomas have diffuse prolongations in the tumoral stroma, the neoplastic cells being in direct contact with stromal cells or inflammatory cells, an aspect that might explain the more intense inflammatory reaction in these types of carcinomas. Also, there was observed that most BCCs had a tendency to develop a denser stroma, sometimes with a desmoplastic aspect, while CSSs most often presented a lax stroma allowing the accumulation of abundant peritumoral inflammatory infiltrates.

The evaluation of the main types of inflammatory cells in the tumoral stroma of NMSCs was performed by using certain specific immunohistochemical markers. The use of anti-CD3 antibody allowed us to remark that in SCCs, the peritumoral infiltrate contained a higher number of CD3+ T-lymphocytes (Figure 5), in comparison to BCCs, where CD3+ T-lymphocytes were more reduced (Figure 6). In SCCs, there were observed CD3+ T-lymphocytes among the tumoral cells, in a close relation to these (Figures 7 and 8), which makes us believe that these cells are directly involved in inducing the immune tolerance. In poorly and moderately differentiated SCCs, the number of CD3+ T-lymphocytes present among the tumoral cells was much higher than in well-differentiated SCCs.

B-lymphocytes, highlighted by using the anti-CD20 antibody, were less numerous in the inflammatory infiltrate of the tumoral stroma of NMSCs, in comparison to T-lymphocytes. Their distribution was completely heterogeneous from one area to another of the tumoral stroma. Similarly to T-lymphocytes, most B-lymphocytes were identified in the tumoral stroma of SCCs, especially of poorly and moderately differentiated SCCs (Figure 9); in BCCs, the number of B-lymphocytes was very low

(Figure 10). In SCCs, there were observed CD20+ B-lymphocytes in close relation to neoplastic cells.

In the skin immune system, the cells of the macrophage system play an essential part in detecting and presenting the antigens that reach the structures, as well as in the phagocytizing of pathogenic agents, antigens, changed self-molecules or other non-self cells. Their role in carcinogenesis is still unclear. In our study, highlighting macrophages in NMSCs was performed by using the anti-CD68 antibody. We observed that the macrophage distribution, similar to T- and B-lymphocytes, was a heterogeneous one in the peritumoral stroma. Most macrophages were highlighted in SCCs, especially the poorly and moderately differentiated ones (Figure 11). Also, there were frequently highlighted intratumoral macrophages, in a direct contact with neoplastic cells (Figure 12).

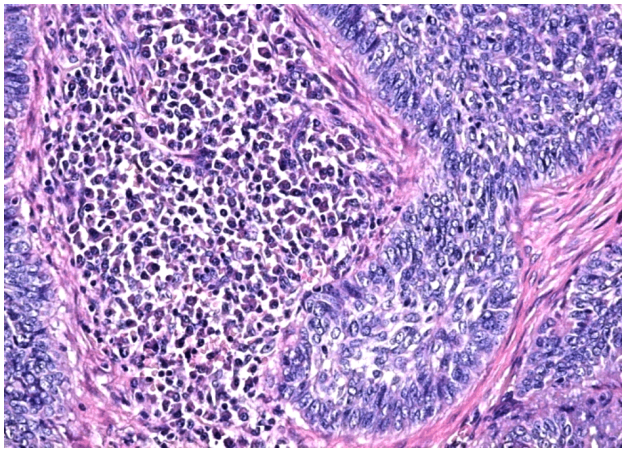

Figure 1 – ***Basal cell carcinoma with intense peritumoral inflammatory reaction (HE staining, ×200).***

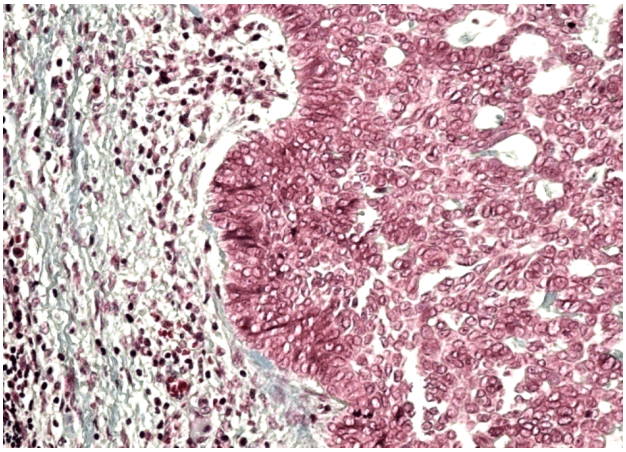

Figure 2 – ***Basal cell carcinoma with low peritumoral inflammatory reaction (GS trichrome staining, ×200).***

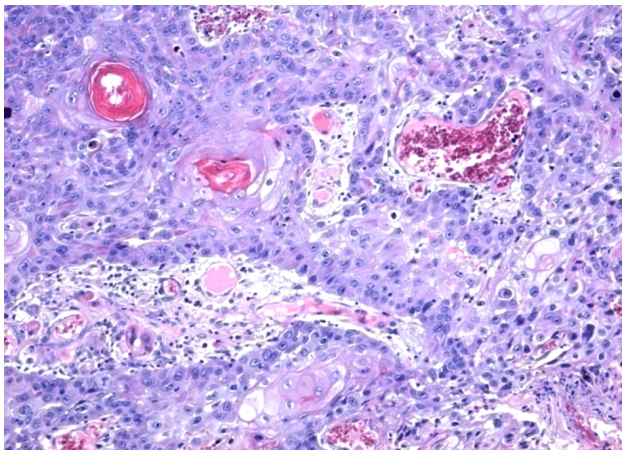

Figure 3 – ***Squamous cell carcinoma with low peritumoral inflammatory reaction (HE staining, ×100).***

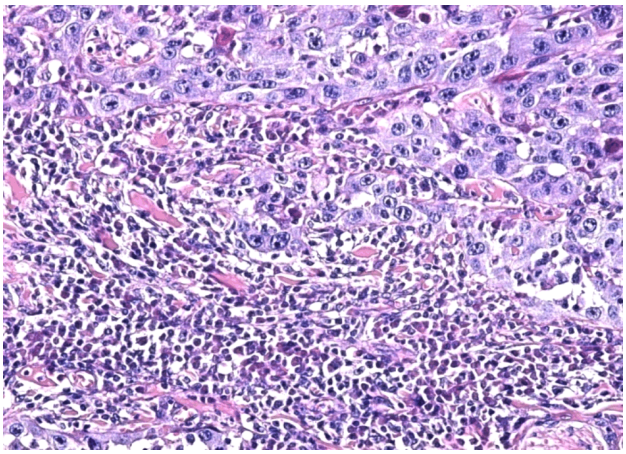

Figure 4 – ***Image of poorly differentiated squamous cell carcinoma with intense peritumoral inflammatory reaction (HE staining, ×200).***

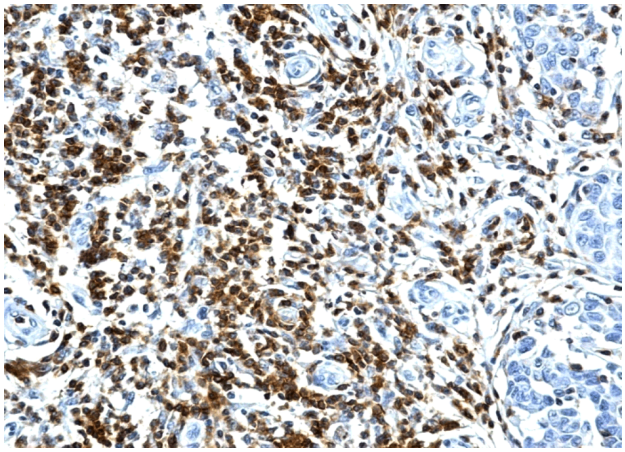

Figure 5 – ***Poorly differentiated squamous cell carcinoma with stroma strongly infiltrated with inflammatory cells where CD3+ T-lymphocytes are well represented (Anti-CD3 antibody immunomarking, ×200).***

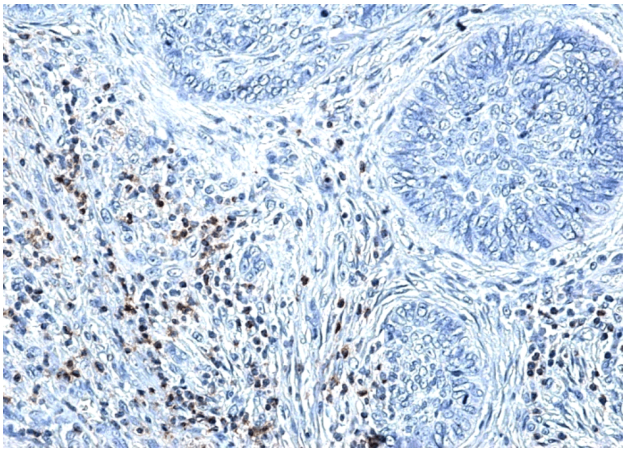

Figure 6 – ***BCC image with moderate inflammatory reaction, CD3+ T-lymphocytes are in low number (Anti-CD3 antibody immunomarking, ×200).***

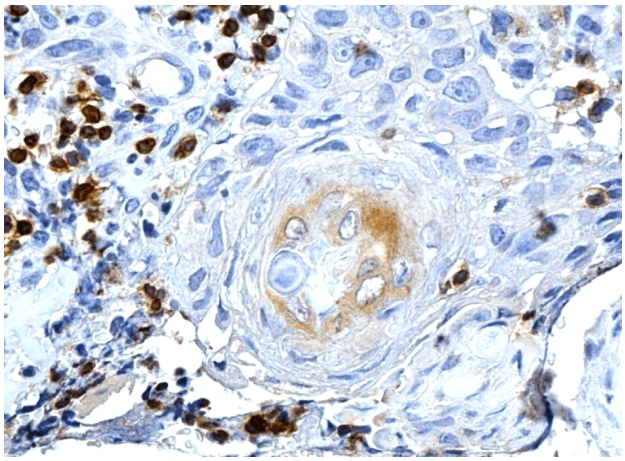

Figure 7 – ***Image of well-differentiated SCC (with keratosis pearls) where there may be observed the presence of T CD3+ lymphocytes in a close relation with tumor cells (Anti-CD3 antibody immunomarking, ×400).***

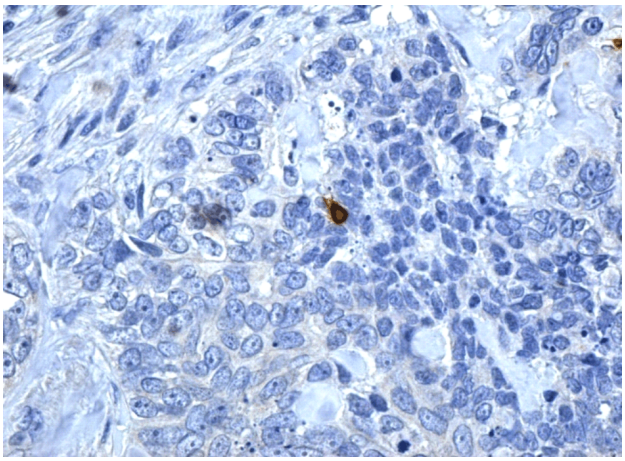

Figure 8 – ***Poorly differentiated SCC with T-lymphocytes in contact with tumor cells (Anti-CD3 antibody immunomarking, ×400).***

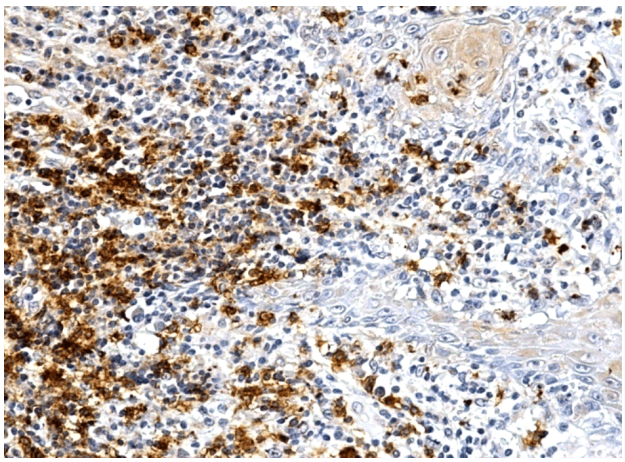

Figure 9 – ***Image of moderately differentiated SCC with intense peritumoral inflammatory reaction, with well-represented T-lymphocytes (Anti-CD20 antibody immunomarking, ×200).***

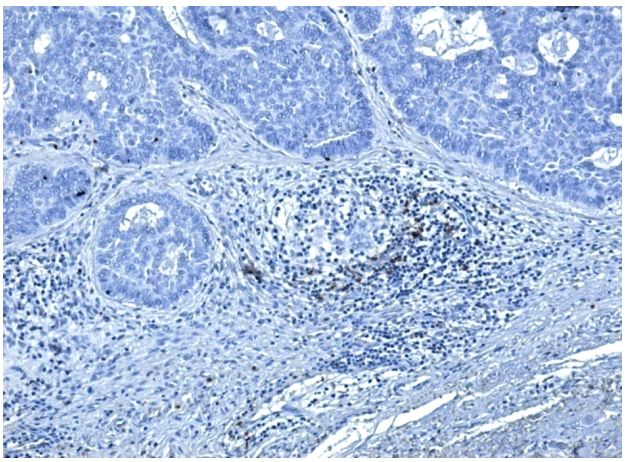

Figure 10 – ***BCC with moderate inflammatory reaction, with rare B-lymphocytes in tumor stroma (Anti-CD20 antibody immunomarking, ×100).***

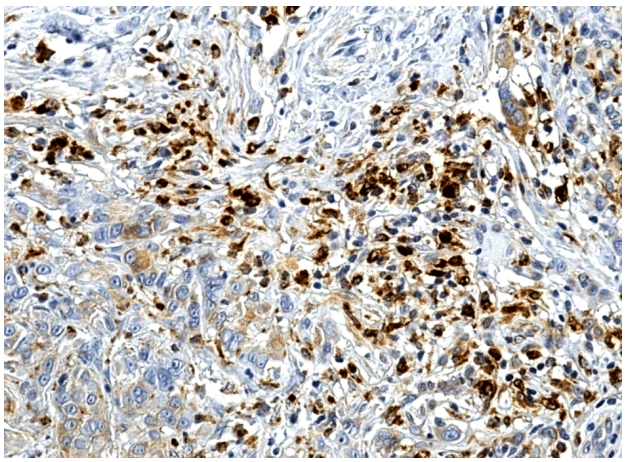

Figure 11 – ***Image of moderately differentiated SCC with numerous macrophages present in the tumor stroma (Anti-CD68 antibody immunomarking, ×200).***

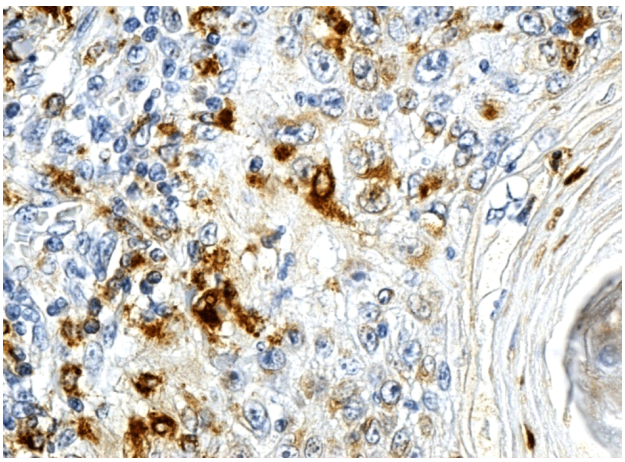

Figure 12 – ***SCC with numerous intratumoral macrophages (Anti-CD68 antibody immunomarking, ×400).***

On the contrary, in BCCs, the macrophage reaction was moderate or reduced (Figure 13).

The evaluation of mast cells reaction in NMSCs was performed by using the anti-tryptase antibody. In our study, in SCCs there was identified a high number of mast cells in the peritumoral inflammatory infiltrate, and also in the intratumoral one (Figures 14 and 15). In BCCs, the number of mast cells identified in the chronic inflammatory infiltrate in the peritumoral stroma was reduced (Figure 16).

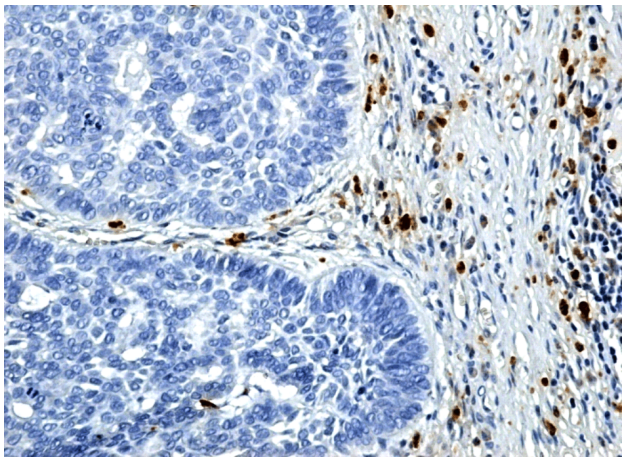

Figure 13 – ***Image of BCC with low number of macrophages in the peritumoral chronic inflammatory infiltrate (Anti-CD68 antibody immunomarking, ×200).***

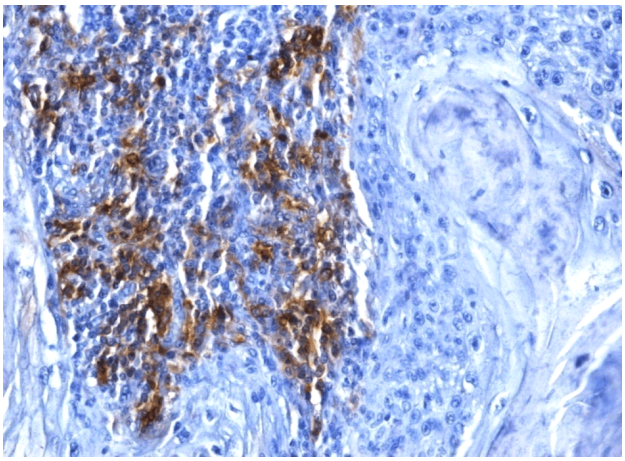

Figure 14 – ***Moderately differentiated SCC with intense inflammatory reaction in the peritumoral stroma and a high number of mast cells (Anti-tryptase immunomarking, ×200).***

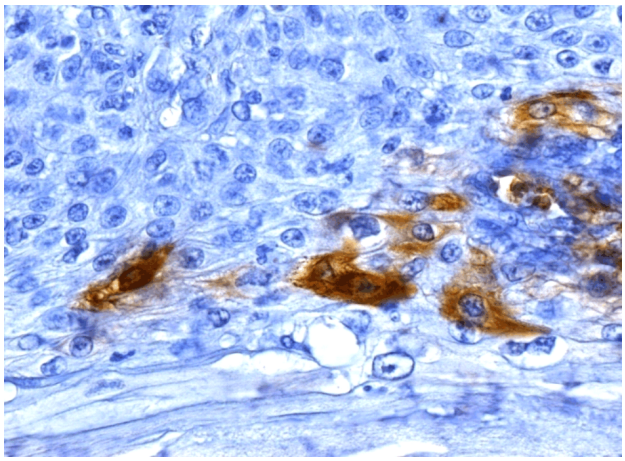

Figure 15 – ***Poorly differentiated SCC with numerous intratumoral mast cells (Anti-tryptase immunomarking, ×400).***

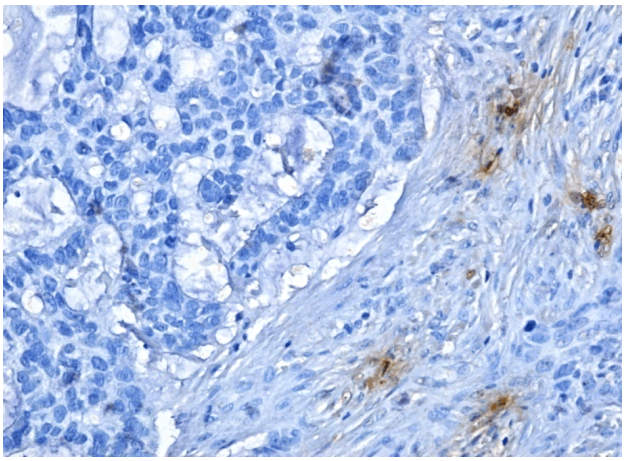

Figure 16 – ***Image of BCC with a low peritumoral inflammatory reaction, with a low number of mast cells localized in the peritumoral stroma (Anti-tryptase immunomarking, ×200).***

Discussion

NMSCs represent an extremely frequent pathology world widely, mainly in white skin persons. According to some studies, the global incidence of NMSCs increased from 1960 by a rate of 3–8% every year [3]. Most studies show that the main factor involved in the etiopathogeny of NMSCs is represented by the UV radiations of the sun, which cause a multitude of lesions of the keratinocyte genetic material, especially deletions and genetic mutations, thus activating the oncogenes. Because of solar UV exposure, the incidence of NMSCs is correlated to the geographical latitude [2], while the most frequent lesions appear on the skin areas exposed to solar radiations. In our study, of the 73 NMSCs, 70 (95.89%) were localized on skin areas that were exposed to solar radiations (61 on the face and nine on the hands), which confirms the hypothesis that the main etiopathogenic factor of NMSCs is represented by solar UV radiations.

Relatively recent data show that UV A radiations with a wavelength of 320–400 nm and UV B radiations with a wavelength of 280–320 nm act as initiators and promoters of carcinogenesis, by a direct harming of the keratinocyte DNA. Moreover, they cause skin immunosupression, chronic inflammation and oxygen-reactive species favoring carcinogenesis [3, 13, 16–20]. There should be noted that UV-caused lesions on DNA are cumulative in time and explain the onset of skin cancer, especially in the elderly.

Other risk factors mentioned in various epidemiological studies, except UV radiations, are represented by old age, immunosupression (induced or acquired), chronic inflammatory disease, human papillomavirus infections, genetic diseases, etc. [21]. Although the skin has numerous defense mechanisms against UV radiations, cancer chemical agents, oncogene viruses [22] or pathogenic bacteria, it is the headquarters of one of the most frequent neoplasia: non-melanoma skin cancer.

We must show that the immune system of the skin is extremely complex, being formed of a network of effectors of cellular and humoral immunity that cooperate against physical, chemical or biological attacks. The cell populations that cooperate for preserving the homeostasis of the skin organ are represented by keratinocytes, antigen presenting dendritic cells, cells of the macrophage system, lymphocytes, granulocytes, mast cells and vascular endothelial cells [23]. These cell populations, of an immune and non-immune origin, communicate among them through

some specific mediators, such as cytokines, chemokines, immunoglobulins, neuropeptides, prostaglandins, free radicals, anti-microbial peptides, etc. Regarding the process of tumorigenesis, Paul Ehrlich, ever since 1909, suggested that the immune system may provide protection against cancer [24], while other studies consider that inflammatory processes often facilitate the cancer development [25, 26]. In other words, the peritumoral inflammatory reaction may be a defense mechanism, and also a factor favoring the development of neoplasia.

Most studies consider that the immune system of the skin plays the main part in developing certain mechanisms of anti-tumoral monitoring and skin carcinogenesis blocking, but when its defense capacity is obsolete, it may favor the development of skin tumors [27].

In our study, all cases of NMSCs presented a chronic inflammatory reaction in the peritumoral stroma, more or less intense mainly formed of mononuclear round cells. These pathological aspects show that between NMSCs and the immune system there is a very close relation. The heterogeneous aspect of the peritumoral chronic inflammatory infiltrate may be due to the presence of a varied quantity of biochemical mediators of inflammation produced both by the immune system cells and by the tumoral cells. Moreover, NMSCs by their development, they destroy the natural defense barriers of the skin, thus allowing the entrance of some microorganisms (saprophyte bacteria, viruses) or of other antigens on skin surface in its depth, thus amplifying the inflammatory reaction and its heterogeneous aspect. Also, in evaluating the variability of the peritumoral inflammatory reaction in NMSCs, we must also take into consideration the individual reactivity.

The molecular mechanisms determining the onset of peritumoral inflammatory reaction are not completely understood. Some studies mention that UV radiations produce reactive oxygen species (ROS) at skin level that alter the deoxyribonucleic acid (DNA), harms the cellular membranes, alters enzyme systems, finally leading to the deterioration of epidermal keratinocytes and of skin conjunctive tissue [28, 29]. The emergence of certain cellular and molecular changes in the skin structure activates the immune system, which leads to the accumulation of macrophages, lymphocytes, granulocytes and mast cells. The inborn immune system cells, residing in the skin tissues react by secreting various mediators, which, in their turn, lead to the activation of resident mesenchymal cells (fibroblasts, endothelial cells), thus involving changes of the conjunctive tissue and driving the cells of the adaptive immune system [30]. When the injury is chronic, inflammation, which was initially an acute response, becomes chronic, thus creating a favorable environment for the onset of carcinogenesis [22, 31]. At present, chronic inflammation is accepted as a distinctive sign of cancer development, where the immune system cells exert both pro-tumoral and anti-tumoral activities, according to their state of activation and their microenvironment [25, 32, 33].

In our study, the peritumoral inflammatory reaction was more intense in SCCs in comparison to BCCs. This difference may be correlated with the more aggressive character of SCCs compared to BCCs. Also, in SCCs we identified the presence of a more abundant inflammatory infiltrate in poorly differentiated carcinomas, in comparison to well-differentiated SCCs. According to some studies [34], the complexity of peritumoral inflammatory infiltrates depends largely on the tissue where the tumor develops, the tumor stage and the tumoral differentiation degree.

In our study, by evaluating the presence of T- and B-lymphocytes in the inflammatory infiltrate, we observed that T-lymphocytes are prevailing, which shows that cellular immunity is intensely involved in carcinogenesis. Similar data were reported in other types of cancer [35, 36]. It is well known the fact that cytotoxic (CD8+) T-lymphocytes are responsible for tumor regression, acting upon tumoral cells through the gamma interferon (IFN-γ). Still, some tumoral cells often escape the aggressiveness of T-cells by reducing the expression of IFN-γ receptors [22], thus allowing tumor multiplication and growth.

The cells of the monocyte-macrophage system, immunohistochemically marked by using the anti-CD68 antibody, were in a relatively higher number in the inflammatory infiltrate of NMSCs, but with a heterogeneous distribution. Macrophages represent an important group of non-specific immune cells, originating in the blood monocytes, capable of infiltrating islands of tumor cells or the peritumoral stroma. Recent studies showed that these macrophages, called tumor-associated macrophages (TAMs) are two phenotypes with opposite functions: the M1 phenotype, which exerts a cytotoxic effect on cancer cells (by releasing reactive oxygen or nitrogen species and inflammatory cytokines) and the M2 phenotype that enables the development of cancer cells (by releasing some growth factors that promote tumor mass growth and vascularization) [37–41].

The study of mast cells reaction in NMSCs allowed us to remark that their density was higher in SCCs, compared to BCCs. Moreover, the thorough examination with strong microscopic objectives showed that around the mastocytes there were numerous tryptase positive granules, thus proving their secretor cell characteristic, including in the tumoral stroma. Their role in carcinogenesis is still incompletely known. Mast cells were among the first cells of the immune system considered as playing a protumoral part [42]. In skin cancers, mast cells, under the influence of UV radiations, secrete a multitude of mediators, proangiogenic factors and biochemical compounds that are involved in the remodeling of skin tissue, thus favoring the development of tumor cells [43].

We consider that the relations among the immune system cells and NMSCs are extremely complex. Tumor cells are capable of synthesizing and secreting a multitude of biochemical products through which they alter the surrounding environment or act upon the immune system cells, missing the immune defense. There was proven that cancer cells may secrete immunosupressor cytokines, such as interleukin (IL)-10 and transforming growth factor-beta (TGF-β), factors by which they induce immune tolerance [44, 45]. In their turn, the immune system cells synthesize and secrete numerous growth factors (epidermal growth factor – EGF), TGF-β, tumor necrosis factor-alpha (TNF-α), fibroblast growth factors (FGFs), interleukins, chemokines, histamine and heparins, various classes of proteolytic enzymes, that may selectively

break down some proteins and glycoproteins and may thus alter the structure and function of the extracellular matrix, thus facilitating or inhibiting the process of carcinogenesis [30, 46]. This vast variety of biochemical factors make that peritumoral inflammatory reaction be in a continuous remodeling and present diverse microscopic aspects [47].

The presence of immune system cells (T-lymphocytes, macrophages, mastocytes) among tumor cells, in direct contact to these, makes us believe that between the two categories of cells there may arise intercellular communication mechanisms different from the already known paracrine signaling mechanisms.

Together with other authors [48], we consider that in NMSCs the interactions between the immune system cells and tumor cells are complex ones, and we are still trying to understand them.

Conclusions

The study of the inflammatory reaction in the 73 cases of NMSCs showed a heterogeneous distribution of the immune system cells on the section surface of the same tumor, probably because of a very high variability of the antigenic structure distribution and/or the cellular signaling factors. The peritumoral inflammatory reaction was more intense in SCCs in comparison to BCCs and in poorly differentiated carcinomas, in comparison to the well-differentiated ones. By evaluating the presence of T- and B-lymphocyes in the peritumoral inflammatory infiltrate, we observed that T-lymphocytes are more numerous, which shows that cellular immunity is intensely involved in skin carcinogenesis. The presence of immune system cells (T-lymphocytes, macrophages, mastocytes) among the tumor cells, in direct contact to these, makes us believe that between the two-cell categories there may arise intercellular communication mechanisms different from the paracrine signaling mechanisms.

Conflict of interests

The authors declare that they have no conflict of interests.

Author contribution

Ioan Avrămoiu and Ileana Octavia Petrescu equally contributed to the manuscript.

References

[1] Almahroos M, Kurban AK. Ultraviolet carcinogenesis in non-melanoma skin cancer. Part I: incidence rates in relation to geographic locations and in migrant populations. Skinmed, 2004, 3(1):29–35; quiz 35–36.

[2] Ramos J, Villa J, Ruiz A, Armstrong R, Matta J. UV dose determines key characteristics of nonmelanoma skin cancer. Cancer Epidemiol Biomarkers Prev, 2004, 13(12):2006–2011.

[3] Tufaro AP, Chuang JC, Prasad N, Chuang A, Chuang TC, Fischer AC. Molecular markers in cutaneous squamous cell carcinoma. Int J Surg Oncol, 2011, 2011:231475.

[4] Xiang F, Lucas R, Hales S, Neale R. Incidence of non-melanoma skin cancer in relation to ambient UV radiation in white populations, 1978–2012: empirical relationships. JAMA Dermatol, 2014, 150(10):1063–1071.

[5] Rogers HW, Weinstock MA, Harris AR, Hinckley MR, Feldman SR, Fleischer AB, Coldiron BM. Incidence estimate of nonmelanoma skin cancer in the United States, 2006. Arch Dermatol, 2010, 146(3):283–287.

[6] Nowotarski SL, Feith DJ, Shantz LM. Skin carcinogenesis studies using mouse models with altered polyamines. Cancer Growth Metastasis, 2015, 8(Suppl 1):17–27.

[7] Tânţu MM, Belu E, Man GM, Manu D, Rogozea LM, Stocheci CM, Bălăşoiu AT, Stănescu C, Domnariu CD. Prevalence and histopathological types of skin carcinomas in Arges County, Romania. Rom J Morphol Embryol, 2014, 55(3):803–809.

[8] Bălăşoiu AT, Mănescu MR, Bălăşoiu M, Avrămoiu I, Pirici I, Burcea M, Mogoantă L, Mocanu CL. Histological and immunohistochemical study of the eyelid basal cell carcinomas. Rom J Morphol Embryol, 2015, 56(2 Suppl):803–810.

[9] Patel RV, Frankel A, Goldenberg G. An update on non-melanoma skin cancer. J Clin Aesthet Dermatol, 2011, 4(2): 20–27.

[10] Fransen M, Karahalios A, Sharma N, English DR, Giles GG, Sinclair RD. Non-melanoma skin cancer in Australia. Med J Aust, 2012, 197(10):565–568.

[11] Mudigonda T, Pearce DJ, Yentzer BA, Williford P, Feldman SR. The economic impact of non-melanoma skin cancer: a review. J Natl Compr Canc Netw, 2010, 8(8):888–896.

[12] Pleasance ED, Cheetham RK, Stephens PJ, McBride DJ, Humphray SJ, Greenman CD, Varela I, Lin ML, Ordóñez GR, Bignell GR, Ye K, Alipaz J, Bauer MJ, Beare D, Butler A, Carter RJ, Chen L, Cox AJ, Edkins S, Kokko-Gonzales PI, Gormley NA, Grocock RJ, Haudenschild CD, Hims MM, James T, Jia M, Kingsbury Z, Leroy C, Marshall J, Menzies A, Mudie LJ, Ning Z, Royce T, Schulz-Trieglaff OB, Spiridou A, Stebbings LA, Szajkowski L, Teague J, Williamson D, Chin L, Ross MT, Campbell PJ, Bentley DR, Futreal PA, Stratton MR. A comprehensive catalogue of somatic mutations from a human cancer genome. Nature, 2010, 463(7278):191–196.

[13] Kim Y, He YY. Ultraviolet radiation-induced non-melanoma skin cancer: regulation of DNA damage repair and inflammation. Genes Dis, 2014, 1(2):188–198.

[14] Okada F. Inflammation and free radicals tumor development and progression. Redox Rep, 2002, 7(6):357–368.

[15] Demaria S, Pikarsky E, Karin M, Coussens LM, Chen YC, El-Omar EM, Trinchieri G, Dubinett SM, Mao JT, Szabo E, Krieg A, Weiner GJ, Fox BA, Coukos G, Wang E, Abraham RT, Carbone M, Lotze MT. Cancer and inflammation: promise for biologic therapy. J Immunother, 2010, 33(4):335–351.

[16] Ullrich SE. Photoimmune suppression and photocarcinogenesis. Front Biosci, 2002, 7:d684–d703.

[17] de Gruijl FR. Ultraviolet radiation and tumor immunity. Methods, 2002, 28(1):122–129.

[18] Ichihashi M, Ueda M, Budiyanto A, Bito T, Oka M, Fukunaga M, Tsuru K, Horikawa T. UV-induced skin damage. Toxicology, 2003, 189(1–2):21–39.

[19] Kunisada M, Sakumi K, Tominaga Y, Budiyanto A, Ueda M, Ichihashi M, Nakabeppu Y, Nishigori C. 8-Oxoguanine formation induced by chronic UVB exposure makes Ogg1 knockout mice susceptible to skin carcinogenesis. Cancer Res, 2005, 65(14):6006–6010.

[20] Katiyar SK. Hsp90 inhibitor can inhibit UV carcinogenesis. J Invest Dermatol, 2015, 135(4):945–947.

[21] Ulrich C, Schmook T, Sachse MM, Sterry W, Stockfleth E. Comparative epidemiology and pathogenic factors for non-melanoma skin cancer in organ transplant patients. Dermatol Surg, 2004, 30(4 Pt 2):622–627.

[22] Medler TR, Coussens LM. Duality of the immune response in cancer: lessons learned from skin. J Invest Dermatol, 2014, 134(e1):E23–E28.

[23] Woods GM, Malley RC, Muller HK. The skin immune system and the challenge of tumour immunosurveillance. Eur J Dermatol, 2005, 15(2):63–69.

[24] Rangwala S, Tsai KY. Roles of the immune system in skin cancer. Br J Dermatol, 2011, 165(5):953–965.

[25] Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. Cancer Cell, 2012, 21(3):309–322.

[26] Briso EM, Guinea-Viniegra J, Bakiri L, Rogon Z, Petzelbauer P, Eils R, Wolf R, Rincón M, Angel P, Wagner EF. Inflammation-mediated skin tumorigenesis induced by epidermal c-Fos. Genes Dev, 2013, 27(18):1959–1973.

[27] Bulman A, Neagu M, Constantin C. Immunomics in skin cancer – improvement in diagnosis, prognosis and therapy monitoring. Curr Proteomics, 2013, 10(3):202–217.

[28] Schwarz T. [Ultraviolet radiation – immune response]. J Dtsch Dermatol Ges, 2005, 3(Suppl 2):S11–S18.
[29] Fartasch M, Diepgen TL, Schmitt J, Drexler H. The relationship between occupational sun exposure and non-melanoma skin cancer: clinical basics, epidemiology, occupational disease evaluation, and prevention. Dtsch Arztebl Int, 2012, 109(43): 715–720.
[30] Balkwill F, Charles KA, Mantovani A. Smoldering and polarized inflammation in the initiation and promotion of malignant disease. Cancer Cell, 2005, 7(3):211–217.
[31] Coussens LM, Werb Z. Inflammation and cancer. Nature, 2002, 420(6917):860–867.
[32] de Visser KE, Eichten A, Coussens LM. Paradoxical roles of the immune system during cancer development. Nat Rev Cancer, 2006, 6(1):24–37.
[33] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell, 2011, 144(5):646–674.
[34] Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature, 2008, 454(7203):436–444.
[35] Ruffell B, Au A, Rugo HS, Esserman LJ, Hwang ES, Coussens LM. Leukocyte composition of human breast cancer. Proc Natl Acad Sci U S A, 2012, 109(8):2796–2801.
[36] Mogoantă SŞ, Lungu C, Ilie C, Albu DF, Totolici B, Neamţu C, Mitruţ P, Dogaru CA, Turculeanu A. Peritumoral inflammatory reaction in colon cancer. Histological and immunohistochemical study. Rom J Morphol Embryol, 2014, 55(4):1429–1435.
[37] Kosaka T, Kuwabara M, Endo A, Tamaguchi H, Koide F. Expression of arginase by mouse myeloid leukemic cell differentiation *in vitro* induced with tumor necrosis factor. J Vet Med Sci, 1991, 53(1):53–57.
[38] Lewis CE, Pollard JW. Distinct role of macrophages in different tumor microenvironments. Cancer Res, 2006, 66(2):605–612.
[39] Sica A, Larghi P, Mancino A, Rubino L, Porta C, Totaro MG, Rimoldi M, Biswas SK, Allavena P, Mantovani A. Macrophage polarization in tumour progression. Semin Cancer Biol, 2008, 18(5):349–355.
[40] Mantovani A, Sica A, Allavena P, Garlanda C, Locati M. Tumor-associated macrophages and the related myeloid-derived suppressor cells as a paradigm of the diversity of macrophage activation. Hum Immunol, 2009, 70(5):325–330.
[41] Zhang M, He Y, Sun X, Li Q, Wang W, Zhao A, Di W. A high M1/M2 ratio of tumor-associated macrophages is associated with extended survival in ovarian cancer patients. J Ovarian Res, 2014, 7:19.
[42] Coussens LM, Raymond WW, Bergers G, Laig-Webster M, Behrendtsen O, Werb Z, Caughey GH, Hanahan D. Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis. Genes Dev, 1999, 13(11):1382–1397.
[43] Sarchio SN, Kok LF, O'Sullivan C, Halliday GM, Byrne SN. Dermal mast cells affect the development of sunlight-induced skin tumours. Exp Dermatol, 2012, 21(4):241–248.
[44] Beck C, Schreiber H, Rowley D. Role of TGF-beta in immune-evasion of cancer. Microsc Res Tech, 2001, 52(4):387–395.
[45] Kawamura K, Bahar R, Natsume W, Sakiyama S, Tagawa M. Secretion of interleukin-10 from murine colon carcinoma cells suppresses systemic antitumor immunity and impairs protective immunity induced against the tumors. Cancer Gene Ther, 2002, 9(1):109–115.
[46] Lu P, Takai K, Weaver VM, Werb Z. Extracellular matrix degradation and remodeling in development and disease. Cold Spring Harb Perspect Biol, 2011, 3(12).
[47] Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science, 2011, 331(6024):1565–1570.
[48] Dannenmann SR, Thielicke J, Stöckli M, Matter C, von Boehmer L, Cecconi V, Hermanns T, Hefermehl L, Schraml P, Moch H, Knuth A, van den Broek M. Tumor-associated macrophages subvert T-cell function and correlate with reduced survival in clear cell renal cell carcinoma. Oncoimmunology, 2013, 2(3):e23562.

Corresponding author
Marius Eugen Ciurea, Professor, MD, PhD, Department of Plastic Surgery and Reconstructive Microsurgery, University of Medicine and Pharmacy of Craiova, 2 Petru Rareş Street, 200349 Craiova, Romania; Phone +40745–614 248, e-mail: meciurea@gmail.com

Received: October 3, 2015

Accepted: December 14, 2016