Mast cells and dendritic cells in basal cell carcinoma

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Abstract
Basal cell carcinoma (BCC) is a very frequent skin malignancy, with slow evolution and rare metastases. Host tissues react to tumor invasion through complex inflammatory response, comprising varied inflammatory cells. We assessed the expression of mast cells and dendritic cells in 37 archived formalin-fixed paraffin-embedded tissue samples of BCC from the oral and maxillofacial region by means of immunohistochemical (IHC) method, using the SABC (Streptavidin–Biotin Complex) indirect triadal technique for CD117 and S100 markers. Undetermined cases were eliminated. Mast cells were found in great number at the periphery and in between the tumor islands, the positivity to CD117 being high in three cases, moderate in 11 cases and low in 16 cases. Dendritic cells were also found within the tumor stroma, but they penetrated deep inside the tumor nests. The positivity to S100 was high in one of the 20 conclusive BCC cases, moderate in seven cases and low in nine cases. Three cases were negative to S100. The characteristic location of dendritic cells prove their role as antigen-receptor cells while mast cells might play dual roles in tumor biology.

Keywords: basal cell carcinoma, mast cells, dendritic cells, CD117, S100.

Introduction
Basal cell carcinoma (BCC) is the most frequent skin cancer, arising from the basal cells of the epidermis and pilosebaceous units. It is most often located in sun-exposed skin, especially the face, it has slow growth rate and local destructive potential. The clinical and microscopic features are polymorphous. The histological appearance includes undifferentiated (nodular and infiltrative subtypes) and differentiated forms. The differentiation is slight and made towards the cutaneous appendages of hair (keratotic BCC), sebaceous glands (BCC with sebaceous differentiation), apocrine/eccrine glands (adenoid BCC). Many BCCs display both undifferentiated and differentiated areas. There is no difference in the rate of growth between the two groups of BCCs. Other microscopic types are morpheaform (sclerodermiform), fibroepitelialoma, superficial, adamantinoid, granular, clear-cell, and with matricial differentiation [1–3]. Certain risk locations and histopathological subtypes may trigger deep invasion of tissue structures, with progression toward vital organs. This situation is detected in BCCs near face cavities (mouth, nose, ear, eyes) and in deeply penetrating BCC subtypes, such as infiltrative, morpheaform, micronodular or their combination [4].

Host tissues react to the tumoral invasion by various mechanisms, such as intense inflammatory reaction, comprising dense lymphocytic infiltrates, mast cells, dendritic cells, plasmocytes, neutrophils, eosinophils, etc. Mast cells are big, mobile connective cells, with round, oval or uneven shaped and a diameter around 25 μm. They take part in a large palette of biological activities: phagocytosis, antigen processing, cytokines production, release of physiological mediators (such as histamine, heparin, proteoglycans, proteases, leukotriens, prostaglandines, etc.) [5]. On their surface, they bear adhesion molecules, immune response receptors, which allow them to react to numberless specific and non-specific stimuli [6]. Due to such wide biological features and to their strategic location near blood vessels, nerves, inflamed or neoplastic tissues, mast cells play main roles in diverse physiologic, immunologic and pathologic processes [5, 7–9].

Recent data suggest that mast cells contribute to the tumor genesis of cutaneous malignancies through the following mechanisms:

1. Immunosuppression: Ultraviolet-B radiation, the most important initiator of cutaneous malignancies, activates mast cells. Subsequent to irradiation of the skin, trans-Urocanic acid in the epidermis isomerizes to cis-Urocanic acid, which stimulates the release of neuropeptides from neural c-fibers. These neuropeptides in turn trigger histamine secretion from mast cells, leading to suppression of the cellular immune system.
2. Angiogenesis: Mast cells are the major source of vascular endothelial growth factor (VEGF) in basal cell carcinoma. VEGF is one of the most potent angiogenic factors, which also induces the release of other angiogenic factors across the endothelial cell wall into the matrix. Mast cell proteases reorganize the stroma to facilitate the migration of endothelial cells. In its turn, heparin, the dominant mast cell proteoglycan, assists in blood-borne metastasis.

3. Degradation of extra cellular matrix: Mast cells participate in matrix degradation – process required for tumor spread – through its own proteases, and indirectly through interaction with other cells [10].

Dendritic cells are found in most body tissues, being especially abundant in the skin. At this level, they are named Langerhans cells, and take part in the regulation of keratinocytes mitosis and differentiation and in the skin immune response. After contacting the antigen, they migrate in the lymph nodes, where they encounter the T- and B-cells, which possess the specific receptor for that antigen. Subsequently, they are destroyed through complement activation or by T-killer lymphocytes [11, 12].

The aim of the study was to assess the expression and distribution of mast cells and dendritic cells in skin basal cell carcinoma, through the immunohistochemical markers CD117 and S100.

Material and Methods

Thirty-seven archived formalin-fixed, paraffin-embedded tissue samples of BCC, belonging to the Department of Pathology of Constanța, No. 2 Polyclinic, have been selected for the histopathological analysis, using the standard Hematoxylin and Eosin stain. Tumor biopsies are derived from the oral and maxillofacial region of 37 patients, who underwent surgery in the Constanța Oral and Maxillofacial Surgery Clinic for tumor excision. A modified [13] IHC technique of Hsu SM et al. [14] was performed. Three µm thick sections from formalin-fixed paraffin-embedded specimens were processed by indirect Streptavidin–Biotin Complex method. Briefly, the procedure comprised: deparaffinization in xylene and alcohol series, rehydration, washing in phosphate buffered-saline (PBS), blocking with normal serum, for 20 minutes, incubation with primary antibody overnight then with standard labeled streptavidin antibody biotin (LSAB kit, DAKO, Glostrup, Denmark); washing in carbonate buffer and developing in 3,3’-DAB hydrochloride/H2O2. All specimens were counterstained with Mayer’s Hematoxylin, examined and photographed on a Nikon Eclipse 600 microscope.

Negative control was made by using a primary irrelevant antibody or by replacing the secondary antibody with phosphate buffered-saline (PBS). Positive control was made comparatively with the expression of antibody investigated in the peritumoral cutaneous tissue (positive internal control on slides). In addition, to ensure immunohistochemical accuracy, internal quality control was made, according to a quality guarantee certificate system (ISO 9001/2001). CD117 is a 145–160 kDa cell membrane protein encoded by the c-kit proto-oncogene (chromosome 4q11–12). The protein is a type III tyrosine kinase growth factor receptor for stem cell factor (SCF), also known as mast cell growth factor. CD117 is required for the development and growth of a large number of cells expressing this protein. CD117 is expressed in mast cells, melanocytes and interstitial cells of Cajal. Particularly, mast cells show a strong membrane and cytoplasmic staining [15].

S100 protein is a 21 kDa highly acidic and water-soluble protein first isolated from brain but later shown to be produced by a wide variety of normal and neoplastic cells of mesodermal, neuroectodermal, and epithelial origin. S100 protein may be found in the cell membranes, cytoplasm and nuclei. S100 protein is present in glial cells, Schwann cells and satellite cells (but not perineurial cells), melanocytes, myoepithelial cells, some glandular epithelia (breast, kidney), skeletal and heart muscle cells, fat cells and chondrocytes, and follicular dendritic cells. In our study, it was used to mark Langerhans cells [16].

The distribution of CD117 and S100-positivity has been assessed using the modified Quick score method [17], which takes into account the intensity and distribution of positivity: negative (no staining) = 0; weak (only visible at high magnification) = 1; moderate (readily visible at low magnification) = 2; strong (strikingly positive at low magnification) = 3.

Results

The 37 tumor biopsies belonged to 35 patients, with the mean age of 62 years old and a sex ratio M/F = 2/1. The histopathological subtypes of BCCs of the study batch are shown in Table 1.

Several nodular and nodular infiltrative BCCs also showed areas of cystic transformation, melanin deposits, and adenoid and keratotic differentiation. Of the 37 BCC cases, two were relapses.

Table 1 – Histopathological subtypes of basal cell carcinoma in the study batch

<table>
<thead>
<tr>
<th>Histopathological subtypes</th>
<th>No. of cases</th>
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<tbody>
<tr>
<td>Nodular (N)</td>
<td>13 (35.13%)</td>
</tr>
<tr>
<td>Nodular infiltrative (NI)</td>
<td>12 (32.43%)</td>
</tr>
<tr>
<td>Morphea-like</td>
<td>1 (2.70%)</td>
</tr>
<tr>
<td>Sebaceous differentiation</td>
<td>1 (2.70%)</td>
</tr>
<tr>
<td>Keratotic</td>
<td>2 (5.40%)</td>
</tr>
<tr>
<td>Adenoid</td>
<td>1 (2.70%)</td>
</tr>
<tr>
<td>Trichilemmal differentiation</td>
<td>1 (2.70%)</td>
</tr>
<tr>
<td>Superficial</td>
<td>4 (10.81%)</td>
</tr>
<tr>
<td>Metatypical</td>
<td>2 (5.40%)</td>
</tr>
</tbody>
</table>

Assessment of BCC inflammatory mast cells was made on 30 conclusive cases, out of the initial 37, by help of CD117 IHC marker. Mast cells were present within all tumor stroma, both at the periphery of tumor islands and between them. In certain cases, mast cells were found in the near vicinity of tumor cells, even penetrating the margin of the tumor masses (Figures 1–3).
The positivity to CD117 was moderate in 11 cases and low in 16 cases. Three cases were highly positive and no case was negative to CD117 (Figure 4).

In what concerns the positivity of CD117 as related to BCC subtypes, the more aggressive, nodular infiltrative forms recorded higher scores. Thus, an intense and moderate IHC reaction was seen in eight nodular-infiltrative BCCs, while a weak reaction was noticed only in two cases.

The situation was reverse in nodular BCCs, with only four cases with moderate IHC reaction and nine cases with weak positivity.

The remaining BCC subtypes showed mainly weak positivity to CD117 (Figure 5).

Assessment of BCC dendritic cells was made on 20 conclusive cases. Langerhans cells were located both within the stroma and near the tumor islands frequently penetrating deep inside the tumor masses (Figure 6).

Figure 1 – IHC positive reaction to CD117 in mast cells in BCC stroma, ob. 20×.

Figure 2 – IHC positive reaction to CD117 in mast cells penetrating BCC tumor islands, ob. 20×.

Figure 3 – IHC positive reaction to CD117 in mast cells, ob. 60×.

Figure 4 – Assessment of mast cells distribution in BCC by means of CD117.

Figure 5 – Distribution of CD117-positivity related to BCC subtypes.

Figure 6 – IHC positive reaction to S100 in dendritic cells of keratotic BCC, ob. 20×.
The positivity to S100 was weak in nine cases and moderate in seven cases. Three cases were negative to S100 and only one case was strong positive (Figure 7).

The positivity to S100 related to BCC subtypes showed slight differences between nodular and nodular-infiltrative forms. The seven nodular BCCs found positive to S100 displayed intense IHC reaction in one case, moderate reaction in two cases, weak reaction in three cases and negative reaction in one case.

Nodular-infiltrative BCCs gave a moderate IHC positive reaction in four cases, a weak reaction in another three cases, one case being negative to S100. The remainder of BCCs (keratotic, morpheaform, trichilemmal, superficial and adenoid) showed moderate, low and negative IHC reactions to S100 (Figure 8).

**Discussion**

Our results are in accordance with previous research, which noted that mast cells accumulate at the periphery of skin cancers [18–21]. The increased density of mast cells around many types of tumors is independent of the presence of an inflammatory infiltrate. [22]. Cohen MS and Rogers GS [23] state that the degree of peribasal cell carcinoma inflammation is not correlated with the relative density of mast cells. This fact suggests that mast cells are preferentially recruited near basal cell carcinoma.

Histopathologic studies on BCC in human subjects proved that mast cell density is remarkably high in aggressive BCC subtypes [18, 21, 23, 24]. Our results bear out these studies: we noted intense and moderate positive IHC reaction in eight out of 10 nodular-infiltrative BCCs. In nodular forms, which are less aggressive, we found a low positive reaction in nine cases and a moderate positive reaction in four cases. There is also strong evidence that mast cell accumulation in the peritumoral inflammatory infiltrate contributes to creating a permissive microenvironment to carcinogenesis and metastasis [25–27].

Recent data sustain the auxiliary role of mast cells in the development and progression of skin cancers. These data suggest that mast cells might play opposing roles in tumoral biology, and local conditions might determine mast cells to have promoting or inhibitory effects in tumors.

Thus, certain studies brought evidence on the possibility that mast cells might play dual roles in tumor pathogenesis, and, subsequently, that of BCC. Mast cells own a vast arsenal of mediators, with promoting and inhibitory effects on malignancies [27]. The phenotypic expression of mast cells and their secreting patterns change according to environment.

They have the capacity to secrete individual granules or distinct mediators, selectively [28]. For instance, acidity inhibits allergic degranulation but stimulates production of interleukin-4 (IL-4). IL-6 can be secreted without histamine, in vitro; murine mast cells are able to secrete VEGF without secreting serotonin [29, 30]. Certain studies proved a cytotoxic tumoral effect of mast cells in cutaneous malignancies [18, 31, 32].

Grimbaldeston MA et al. [33] suggest that high prevalence of dermal mast cells constitutes a predisposing factor for BCC development in humans. The authors consider that mast cells function by initiating immunosuppression, thus favorizing a permissive environment for the development of BCC.

Erkiliç S and Erbağcı Z [24], studying mast cells in various BCC subtypes, found the highest values in morpheaform BCC, implying a contributory role for mast cells in the aggressiveness of BCC.

Humphreys TR et al. [34], in a study on 11 BCCs, have found increased numbers of mast cells and dermal dendrocytes in stroma adjacent to tumor nests. No differences in antigen expression were observed between different histological subtypes of BCC.

Yang ML [35], in a study on 17 cases of basal cell carcinoma found that the mast cell number was markedly increased in the dermis near the basal cell carcinoma. There was an increase in the collagen fibers between the carcinoma and dermis tissues, forming a thin membrane around the carcinoma tissue. These findings suggest that the carcinoma-associated antigen may activate the lymphocytes to produce certain lymphokines, which stimulate the proliferation and differentiation of the mast cell precursors. Histamine and other active mediators released from mast cells stimulate fibroblasts to synthesize collagen fibers, which form a thin membrane between the carcinoma...
and dermis. The membrane plays a protective role against tumor dissemination.

Smirnova IO et al. [36] investigated the morpho-functional features of skin mast cells located in the areas subjected to chronic UV-radiation and in the associated basal cell carcinoma with photo-injury. The authors found that chronic UV-damage leads to mast cell hyperplasia as well as activation of their synthetic, absorption and secretory functions. It is suggested that mast cell hyperplasia and the increase of their neuroendocrine activity provide a risk of basal cell carcinoma development.

As far as the presence of dendritic cells is concerned, our results are also similar to those in the specialty literature. For instance, Florell SR et al. [37] made a study in order to investigate if BCC tumor nests contain benign melanocytes and Langerhans [corrected] cells. They found that tumor islands are regularly populated by benign melanocytes and Langerhans [corrected] cells, the last ones being identified in 9 out of the 10 BCC cases studied.

McArdele JP et al. [38], in a study of quantitative evaluation of Langerhans cells in BCC, using the antibody to S100 protein and the indirect immunoperoxidase technique, found an increased density of these cells both within the BCC lesion and in the adjacent non-neoplastic epithelium. The increased Langerhans cells density in the neoplastic epithelium suggests either that they are being retained within the abnormal epithelium for longer periods of time than normal or that they are being actively attracted by factors produced by the neoplastic epithelium. While reduction of intraepithelial Langerhans cells density may allow the initiation of neoplasia, the increased density observed by the authors suggests that at later stages of tumor growth, Langerhans cells may play a functional role in the host response to cutaneous neoplasia.

Murphy GF et al. [39], in a study of lesional and perilesional skin of BCC patients have found marked hyperplasia of Langerhans cells in most cases. Ultrastructurally, Langerhans cells were observed in the dermis and above the basement membrane zone. Apposition of Langerhans cells and indeterminate cells with degenerating and necrotic neoplastic keratinocytes and with exocytotic lymphocytes was frequently encountered. Tumor necrosis areas were frequently found in the near vicinity of exocytotic lymphoid cells. These observations support the idea that an active local immunologic response is related to the biologic behavior of some basal cell carcinomas.

Conclusions

In our study, BCC mast cells were situated in the tumor stroma, sometimes in the near vicinity of tumor nests; the tendency to penetrate the tumor islands was rare.

All 30 conclusive cases under investigation were found positive to CD117; the majority were moderate and weak positive (11 and 16 cases respectively), only three cases being highly positive.

The intensity of the IHC reaction to CD117 was high and moderate with eight out of the 10 nodular-infiltrative BCCs. For nodular BCCs, the IHC reaction was low in nine cases and moderate in four cases. The other BCC subtypes had weak IHC reaction scores.

Dendritic cells in BCC were located both in the stroma and within the tumor nests, reaching their center. This positioning is explained by the fact that dendritic cells play the role of contacting the antigen, which will be subsequently presented to lymphocytes.

The IHC reaction to S100 protein in dendritic cells was positive in 17 out of 20 cases, being moderate and low positive in 16 cases.

References


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