IL-8, IL-8RA (CXCR1) and IL-8RB (CXCR2) expression in pilomatrixcoma

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Abstract

Pilomatrixcoma is a rare benign tumor of the hair follicle matrix cells, which associates during its evolution a foreign body-like inflammatory process. We have investigated three such tumors, two of them displaying a rather poor stroma, while the third was distinctive due to its stroma and large numbers of inflammatory cells infiltrating the tumor. The analysis of IL-8 (interleukin-8), CXCR1 (IL-8RA – IL-8 receptor alpha) and CXCR2 (IL-8RB – IL-8 receptor beta) expression showed that these molecules are present not only in many different types of inflammatory and endothelial cells, but also in several tumor basaloid, transitional and even few ghost cells. Taking into consideration the roles played by IL-8 and its two receptors, CXCR1 and CXCR2, this pattern of expression offers some insights on the potential roles of these molecules in tumor survival, cell proliferation and angiogenesis. Furthermore, given the expression of IL-1 (interleukin-1) and TNF (tumor necrosis factor) receptors by the tumor cells, IL-8 production by such cells might be under the control of these pro-inflammatory cytokines.

Keywords: pilomatrixcoma, IL-8, CXCR1, CXCR2, cytokines.

5 Introduction

Pilomatrixcoma, also known as pilomatrixoma or calcifying epithelioma of Malherbe, is a rare benign skin tumor developed from hair follicle matrix cells [1, 2]. The majority of cases (cca. 60%), are associated with the first two decades of life, females being affected more frequently than males [3, 4]. Usually, the tumor is solitary and its most common localizations are the head, the neck and the upper extremities [5]. Clinical diagnosis can be difficult, but the tissue histological examination easily identifies the particular features of the lesion.

The tumor, frequently limited by a fibrous capsule, appears as islands of cells localized in the dermis. These cells are of epithelial origin with different morphology and features. The cells, arranged in several layers localized in the islands periphery, are called basaloid. They are small, with round nuclei and basophilic cytoplasm. Several basaloid cells, especially those localized in the outer layer, are proliferating cells [1, 2]. In the center of the islands are found the ghost or shadow cells. They are large enucleated cells, with abundant eosinophilic cytoplasm, containing important amounts of keratin. These cells are generated by the keratinization of basaloid cells and their number increases during the evolution of the lesion [2, 6]. In several cases, these two types of cells are separated by layers of so-called transitional cells, with small nuclei and cytoplasm that becomes progressively rich in keratin [1, 2]. Calcification often occurs and sometimes even ossification [7, 8]. The presence of tumor cells is associated with a foreign body-like inflammatory response, the islands being surrounded by a stroma containing usually many blood vessels, multinucleated giant cells and other inflammatory cells [4].

Inflammation is a complex response involving many effector cells of the immune system that accumulate in the inflammatory sites in the attempt to protect the tissues. It can be initiated by many triggers, represented by pathogens and their products, tumor cells or inert tissues, a large variety of factors released by injured cells and involves activated and armed cells able to destroy, as well as cells that contribute by secreting soluble molecules [9].

The chemokines are a family of small cytokines with chemotactic activity. The proteins are divided into two groups based on the presence or the absence of one amino acid in a particular motif containing two cysteine residues: the CC chemokines with no intervening amino acid and CXC chemokines with one such amino acid [10, 11].

IL-8 (interleukin-8) or CXCL8 is a CXC chemokine produced by activated monocytes and macrophages, epithelial and endothelial cells, fibroblasts and neutrophils [12, 13]. It is also known as neutrophil chemotactic factor (NCF), because it induces chemotaxis, primarily of neutrophils, but also of basophils and T-lymphocytes [9–11]. In other target cells such as endothelial cells, macrophages, mast cells and keratinocytes, IL-8 determines various other different effects, such as angiogenesis, phagocytosis, activation, eosinocytes, respiratory burst [9, 14, 15]. All these effects are mediated by the interaction between the chemokine and its two receptors, CXCR1 (IL-8RA – IL-8 receptor alpha) and CXCR2 (IL-8RB – IL-8 receptor beta), members of G-protein-coupled receptors group and expressed by a large variety of cells [16–19].
The recruitment and accumulation of inflammatory cells in the stroma surrounding the islands of tumor cells in pilomatricoma is most probably mediated by many cytokines and chemokines and the aim of our study was to investigate the possible involvement of IL-8 and its two receptors, CXCR1 and CXCR2 in the pilomatricoma-associated inflammation. Additionally, we investigated the presence of IL-1RI (interleukin-1 receptor I), TNFRI (tumor necrosis factor receptor I) and TNFRII (tumor necrosis factor receptor II), as receptors for cytokines such IL-1α, IL-1β and TNF, well known to stimulate the IL-8 secretion [9, 20, 21].

To the best of our knowledge, this is the first investigation of the IL-8 and its receptors expression in pilomatricoma.

Materials and Methods

We investigated three cases of pilomatricoma treated in the Department of Surgery, “Prof. Dr. Nicolae Oblu” Emergency Hospital, Iassy, Romania, in the last two years. The excised tissues were formalin fixed and paraffin embedded. Sections from every case were stained with Hematoxylin and Eosin (HE) for the histological examination.

Immunohistochemistry

Tissue sections (4 μm) were deparaffinized in xylene and rehydrated in a series of graded ethanol solutions. Antigen retrieval was performed by heating the sections, pH 6.1, at 98°C, followed by 5 minutes pre-incubation with 20 minutes in Antigen Retrieval Solution (Dako, Denmark), Antigen retrieval was performed by heating the sections, and rehydrated in a series of graded ethanol solutions.

To investigate the possible involvement of IL-8 and its receptors expression in pilomatricoma.

Results

Histological analysis

The histological examination of the three cases supported the clinical diagnosis but also evidenced several different aspects of the lesions, suggesting different stages of tumor evolution.

In several tumor islands of the first case all types of cells, basaloid, transitional and ghost cells, were well represented (Figure 1), while the stroma, even not very well developed, was dominated by giant cells surrounding many large islands of ghost cells.

The second case displayed numerous large islands of ghost cells, surrounded by a poorly developed stroma, with giant cells, and an extremely low number of basaloid and transitional cells (Figure 2).

The last case was characterized by a small number of islands with basaloid, transitional and ghost cells and a large stroma infiltrated with numerous giant cells, neutrophils, other leukocytes, probably lymphocytes, blood vessels and fibroblasts. Also, many small groups or isolated shadow cells were scattered in the stroma. The inflammatory reaction was present in all three cases, but the third case showed a larger number of giant cells, blood vessels, inflammatory cells and fibroblasts as compared with Case No. 1 or Case No. 2 (Figure 3).

Immunohistochemistry

The IL-8 expression was found in many inflammatory cells, several blood vessels and fibroblasts (Figure 4).

Furthermore, we were able to evidence the IL-8 expression in more than 30% of the basaloid cells, approximately 70% of the transitional cells and even several ghost cells (<5%) in all three cases of pilomatricoma (Figure 5). To the best of our knowledge, this is the first evidence of IL-8 synthesis by the cells constituting these benign skin tumors.

CXCR1 was identified in the basaloid, transitional and in few ghost cells (Figure 6). The positivity percentages were comparable with the IL-8 positivity for every type of the tumor cells. Also, endothelial cells, giant cells, and fibroblasts were CXCR1 positive.

CXCR2 displayed the same pattern of expression as CXCR1 (Figure 7), although the intensity of the labeling was constantly lower, under the same labeling conditions.

The labeling for IL-1 receptor I (IL-1RI) showed positivity in many types of cells scattered within the stroma (Figure 8), but also in 50–60% of the transitional cells and in several basaloid cells (Figure 9).

TNFRII was present in approximately 5% of the basaloid cells, approximately 10% of the transitional cells and few giant cells (Figure 10), while TNFRI was found only in very few giant cells (data not shown).

Due to the small number of the cases, we did not perform any statistical analysis and we did not establish any correlations between the expression of the molecules under investigation. However, we would like to stress that many basaloid cells and the majority of the transitional tumor cells, express simultaneously IL-8 and both its receptors.
Figure 1 – Pilomatricoma – Case No. 1. Tumor islands displaying all three types of pilomatricoma characteristic cells: basaloid, transitional and ghost cells. HE staining, ×100.

Figure 2 – Pilomatricoma – Case No. 2. The ghost cells dominate the lesion, while the stroma is less well represented. HE staining, ×100.

Figure 3 – Pilomatricoma – Case No. 3 characterized by a small number of tumor islands scattered in a stroma extremely abundant, with many inflammatory cells and blood vessels. HE staining, ×200.

Figure 4 – Inflammatory cells, endothelial cells and fibroblasts are IL-8-positive. HRP/DAB (Horseradish peroxidase/3,3’-Diaminobenzidine) immunostaining, ×200.

Figure 5 – IL-8 is present mostly in the transitional cells, but also in basaloid and even in few ghost cells. HRP/DAB immunostaining: (A) ×100; (B) ×200.
Figure 6 – CXCR1 was found in all tumor cells, mostly in the transitional and in the basaloid cells. HRP/DAB immunostaining, ×200.

Figure 7 – CXCR2 has the same pattern of expression as CXCR1. However, the labeling intensity is lower. HRP/DAB immunostaining, ×200.

Figure 8 – IL-1RI is expressed by many cell types present in the stroma surrounding the tumor islands. HRP/DAB immunostaining, ×200.

Figure 9 – Several tumor cells are also positive for IL-1RI. HRP/DAB immunostaining, ×200.

Figure 10 – The anti-TNFRII antibody labels several basaloid and transitional cells. HRP/DAB immunostaining, ×200.

Discussion

Pilomatricoma, a rare and, in most cases, benign tumor of the hair follicle matrix cells, associates a particular foreign body-like inflammation. The most important effector cells seem to be the multinucleated giant cells, responsible for the internalization and destruction of the tumor cells, especially ghost cells [4].

The recruitment of monocytes and macrophages in the tumor tissues, the activation of these cells and their fusion leading to the formation of giant cells is under the control of many factors, some of them soluble molecules, such as cytokines and chemokines [9, 22]. The sources of all these factors are represented by many different types of activated cells in the tumor site. Some of them are resident of the skin, such as macrophages, keratinocytes, mast cells, γδ T-lymphocytes [9, 22]. Other cells have to be recruited from the blood stream or surrounding tissues: neutrophils, additional monocytes and macrophages, NK cells, T- and B-lymphocytes [9, 22]. For this purpose, additional blood vessels have to be generated in a process named angiogenesis. The newly formed vessels will allow many circulating cells to accumulate in the inflammatory site [9, 22].

On the other hand, it is well known that tumors, especially the malignant ones, are able to create their own vascular network in order to support the cells proliferation and tumor growth and to facilitate the cells extravasation and invasion of other tissues [22, 23]. Many such tumor
cells gain the ability to secrete angiogenic factors, such as vascular endothelial growth factors (VEGFs) and IL-8
[24, 25].

IL-8 is secreted by many types of cells, including mast cells, mononuclear, epithelial and endothelial cells stimulated by pro-inflammatory factors [12, 13]. It seems to be a multipotent cytokine and a powerful mediator of inflammation, since it is able to induce so many different effects, such as chemotaxis for neutrophils and T-lymphocytes, activation of various cell types, phagocytosis, exocytosis, respiratory burst and angiogenesis [14, 15, 22].

In the pilomatricomas under investigation, IL-8 expression in the inflammatory and activated endothelial cells was not a surprise, as these cells are described as the most important sources of this cytokine [12, 13]. However, in our study, IL-8 was rather unexpectedly present in all the tumor cell types, basaloid, transitional and, inconsistently, even in ghost cells.

Since the basaloid cells are proliferating cells [26–28], additional blood vessels to support the proliferation and tumor growth are needed and IL-8, an angiogenic factor, might be produced by the tumor cells for this particular purpose [23]. On the other hand, IL-8 production by the tumors’ cells might be a response to stress conditions, such as hypoxia, which, in many cases, is associated with tumors [23, 29].

However, the IL-8 presence in the apoptotic transitional cells and in the ghost cells is difficult to explain. This could be a simple accumulation of the molecule in the cytoplasm of these dying cells, since it was synthesized by their precursors, the basaloid cells. Another explanation is backed by several studies showing that some external signals, for instance those mediated by the TNF (tumor necrosis factor) family members (most notably the Fas/FasL interaction), are able to induce in the same time apoptosis and IL-8 secretion in epithelial target cells [23, 30]. Although we did not find any Fas expression on the tumor cells (data not shown), it may well be possible that many apoptotic cells secrete IL-8, irrespective to the nature of the stimulating signals.

The IL-8 production can be also induced (in tumor epithelial cells as well) by cytokines such as IL-1 [31] or TNF-α [32] and since within the tumor area an increasing number of inflammatory cells tend to accumulate, it is possible that such pro-inflammatory cytokines are secreted by activated cells, especially by macrophages and T-lymphocytes. Such a scenario is supported by our data showing the expression of IL-1RI by the transitional tumor cells and even by several basaloid cells.

Furthermore, our findings regarding the selective presence of TNFRII in a small number of transitional and basaloid cells draw attention as this receptor is known for its ability to transmit signals able to mediate cell survival [33, 34].

The effects of IL-8 upon different target cells are mediated by CXCR1 and CXCR2, two-related high-affinity cell-surface G protein-coupled receptors [16, 17]. CXCR1 seems to interact with two distinct ligands, CXCL8 (IL-8) and CXCL6, while CXCR2 is able to bind many additional molecules, members of the same chemokines family [35]. Both receptors are widely expressed by neutrophils, monocytes and macrophages, endothelial and many epithelial cells, even after malignant transformation [36].

In our study, in all three pilomatricomas, both IL-8 receptors could be evidenced in many different cells, tumor cells, several basaloid, transitional and even a small number of ghost cells, endothelial cells, inflammatory cells, fibroblasts.

It is well known that the IL-8 receptors are capable of mediating various effects once stimulated by their ligands during an immune response or an inflammatory process [16, 35]. The present study does not bring information regarding the functionality of these receptors, so we can only speculate that their presence in endothelial cells, leukocytes and fibroblasts is an indirect evidence for their involvement in mediating IL-8 effects such as chemotaxis, cells’ recruitment and activation, phagocytosis, and angiogenesis [9–11, 14, 15].

The rather unexpected expression of the IL-8 receptors in the tumor cells allowed us to suggest that IL-8-mediated stimulation might be involved in the survival, proliferation and perhaps local invasion of the tumor cells. Many studies demonstrated the IL-8 autocrine stimulation in several malignant tumors such as breast, lung, colon and pancreas cancers [37–40], hence the simultaneously expression of IL-8 and its receptors in pilomatricomas might be a proof for a similar behavior in this benign tumor.

Conclusions

Pilomatricomas are benign tumors that seem to be able to secrete IL-8 and to use the cytokine apparently in an autocrine manner. The interaction between the cytokine and its receptors might be a response of the tumor cells to stress conditions, but might also be involved in the tumor cells survival and angiogenesis. On the other hand, as the tumor development is associated with an immune response and inflammation, IL-8 secretion is definitely playing a role in mediating these processes as well.

Conflict of interests

The authors declare that they have no conflict of interests.

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