FoxP3-positive cells and their contacts with mast cells are highly increased in basal cell carcinoma

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Short Report

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Abstract

The cells of the immune system are thought to contribute to the development of skin cancers through immune evasion. One possible mechanism for this can be the interaction between mast cells and regulatory T cells (Tregs). Therefore, fresh frozen biopsies from the lesional and nonlesional skin of 16 patients with basal cell carcinoma (BCC) were processed for the enzymehistochemical staining of mast cell tryptase, immunohistochemical staining of FoxP3 as well as for the double-staining method to label tryptase+ cells and FoxP3+ (a marker of Tregs) cells on the same cryosection. The cell numbers and apparent morphological contacts (AMCs) between these cell types were counted. The results show a high increase in the number of tryptase+ cells, FoxP3+ cells and AMCs between them in the lesional compared to corresponding nonlesional skin (p<0.0001). Therefore, a theoretical morphological basis is present in the BCC lesion for permitting an immune evasive microenvironment.

Introduction

The incidences of keratinocyte skin cancers, basal cell carcinoma (BCC) and squamous cell carcinoma, have steadily been increasing in Western populations [1], and the ultraviolet (UV) radiation from the sun is the essential risk factor in cutaneous carcinogenesis through immunosuppression and DNA damage [2]. The primary events in carcinogenesis take place in the epidermis, but the cells of the immune system are thought to fail in the immunosurveillance and eradication of mutated cells [3].

The MC_{TC} type (tryptase+, chymase+) of mast cells is increased in the most common skin cancer type, that is, BCC [4]. However, it is not clear whether mast cells play a protumorigenic or antitumorigenic role in this or other skin cancer lesions, as mast cells may have a dual role in the skin immune system depending on the microenvironment [3, 5]. Regulatory T cells (Tregs) are characterized by their expression of the transcription factor forkhead box P3 (FoxP3) and these cells have generally been associated with immunosuppression or tolerance [6]. In a variety of experimental models, the interaction of Tregs with mast cells has been found to be one essential mechanism for immunosuppression, and interleukin-9 from Tregs has been considered to be the essential mediating cytokine [7–9]. In addition, this cellular interaction may be involved in the isomorphic psoriatic lesion, i.e., in the Köbner reaction, through prevention of its development [10]. Therefore, the double-staining technique previously developed for demonstrating tryptase+ mast cells and FoxP3+ cells on the same cryosection [10] was utilized in this study to examine whether these cells interact in BCC. The results show a high increase in the number of these cells and in apparent morphological contacts (AMCs) between them in BCC.

Materials And Methods

Patients

The study included 16 patients with nodular (n = 10) or superficial spreading (n = 6) BCC (5 females, 11 males, age 55–97 years, mean 74). Four mm punch biopsies were taken after local anesthesia with
lidocaine-adrenalin from the lesional and nonlesional (about 2 cm apart from the lesion) skin at varying body sites. The biopsies were immediately embedded in OCT compound (Miles Scientific, Naperville, IL) and frozen for preparing 5-µm-thick cryosections [4, 10].

Histochemical Staining Methods

After fixation of cryosections in ice-cold acetone for 10 min, FoxP3 was stained immunohistochemically using 10 µg/ml mouse monoclonal anti-human FoxP3 antibody (clone 236A/E7) (Abcam, Cambridge, UK), and the bound mAb was visualized with the avidin-biotin-peroxidase (ABC) technique using the Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA) together with 0.05% 3,3′-diaminobenzidine tetrahydrochloride, 0.04% nickel chloride and 0.03% hydrogen peroxide [10]. Unrelated mouse IgG was used as the control. Mast cell tryptase was stained enzymehistochemically using 1 mM Z-Gly-Pro-Arg-4-methoxy-2-naphthylamide as the substrate (Bachem, Bubendorf, Switzerland) and Fast Black K as the chromogen (Sigma, St. Louis, MO) [4]. The numbers of FoxP3+ and tryptase+ cells were counted on separate cryosections using a 0.2x0.2 mm ocular grid in an area of 0.6 mm (depth) x 2.0 mm (width) immediately beneath the lesion or papillary dermis. The results are presented as cells/mm².

The double-staining method for tryptase+ and FoxP3+ cells has previously been developed [10]. Briefly, cryosections were fixed in cold acetone for 10 min followed by blocking with diluted normal horse serum. The sections were treated with anti-FoxP3 mAb and then with biotin-conjugated secondary Ab. Thereafter, tryptase+ cells were identified enzymehistochemically resulting in dark blue to violet mast cells. Finally, FoxP3+ cells were visualized with the ABC-technique that produced black staining. The AMCs between these cells were counted in an area described above, and the results are presented as the percentage of tryptase+ mast cells in AMC with at least one FoxP3+ cell. In addition, the total number of AMCs was counted. Only clearly stained tryptase+, FoxP3+ cells and AMCs were counted.

Statistical Analyses

The results were analyzed using Wilcoxon signed rank test, and p < 0.05 was considered statistically significant.

Results

The results have been summarized in Table 1, and a representative micrograph for AMCs is shown in Fig. 1. The numbers of tryptase+ cells, FoxP3+ cells and AMCs between these cells were highly and significantly increased in the lesional skin compared to the corresponding nonlesional skin in 16 patients with BCC.

Discussion
The clear finding in this study is that the numbers of tryptase* cells, FoxP3* cells and especially the AMCs between them are highly increased in the BCC lesion. Previously, a high number of FoxP3* cells and tryptase* mast cells have been counted in the BCC lesion as well, being in line with this study [4, 11]. However, the present results are descriptive, because it is not known whether these cellular interactions are functional leading to an immunosuppressive state promoting tumor development, even though previous experimental studies [7–9] and correlation analyses between tryptase and FoxP3 in human gastric tumors [12] suggest so. Furthermore, considerable heterogeneity exists among FoxP3*, CD4* Tregs, and the transcription factor can also be expressed to a varying extent and time by other cells, including activated CD4* T cells, CD8* Tregs, natural killer T cells, macrophages, B cells and even some cancer cells [6]. In addition, inflammatory cytokines, including interleukin-1β and IL-6, can antagonize FoxP3 expression or function [6]. Interestingly, TNF-α, a pleiotropic cytokine that can have a dual role depending on the context, can regulate FoxP3 expression and Treg activity [13], and TNF-α immunoreactivity is increased in mast cells in the BCC lesion [14].

In summary, the high increase in FoxP3* cells, tryptase* mast cells and especially in AMCs between them in the BCC lesion suggests that, in theory, the morphological basis is present in the BCC lesion permitting an immune evasive microenvironment. Further experiments are needed to find out whether this cellular interaction is essential for the BCC development.

Declarations

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Contributions

All authors have made substantial contributions to the conception and design of the work; the acquisition, analysis, and interpretation of data; and the writing or reviewing of the manuscript.

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Conflict of interest: The authors report no conflicts of interest.
Ethical statement: The methods were approved by The Ethics Committee of Kuopio University Hospital, Kuopio, Finland, and the study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki.

References


Tables

Table 1. Tryptase+ mast cells, FoxP3+ cells and apparent morphological contacts (AMCs) between these cells in the nonlesional and lesional skin of 16 patients with basal cell carcinoma.

<table>
<thead>
<tr>
<th></th>
<th>Tryptase+ cells (cells/mm²)</th>
<th>FoxP3+ cells (cells/mm²)</th>
<th>AMCs (%)</th>
<th>AMCs (contacts/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesional skin</td>
<td>189 ± 61*</td>
<td>171 ± 123*</td>
<td>13.5 ±10.1*</td>
<td>25.6 ± 21.7*</td>
</tr>
<tr>
<td>Nonlesional skin</td>
<td>86 ± 34*</td>
<td>24 ± 22*</td>
<td>1.2 ±1.2*</td>
<td>1.0 ± 1.0*</td>
</tr>
</tbody>
</table>

The results are expressed as the mean±SD. "*" denotes p<0.0001 between the lesional and nonlesional skin (Wilcoxon signed rank test). “AMCs (%)” denotes the percentage of tryptase+ cells in AMC with at least one FoxP3+ cell.

Figures
Figure 1

FoxP3\(^+\) immunoreactive cells (black) in apparent morphological contacts with tryptase\(^+\) mast cells (violet) in a basal cell carcinoma lesion. The micrograph was taken using a 40x objective.