PhD Thesis

STUDY OF THE INTERACTION BETWEEN STROMA AND EPITHELIUM IN BASAL CELL CARCINOMA. A HISTOLOGICAL AND CLINICO-STATISTICAL APPROACH

- Summary -

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INTRODUCTION

Basal cell carcinoma is an epithelial tumor that develops and depends on the epidermis. Its incidence increases linearly after the age of 40 and its localization is in two thirds of cases in the cervical-facial region.

Basal cell carcinoma is the most frequent epidermal cancer with strictly local malignancy that rarely raises vital prognosis issues. Still some forms have an important destructive potential with consequences sometimes serious from the aesthetical, functional and life quality point of view.

The most important factors in the development of basal cell carcinoma are the genetic and environmental ones.

A remark is due that no other form of cancer is more adaptable to using an efficient prophylaxis as the epidermal carcinomas.

The present paper aims to evaluate the morpho-functional correlation existing between the epithelium and stroma in the basal cell carcinoma, with few clinical and prognosis aspects. Monitoring of the characters modified during the neoplasia process was done through histology techniques, morphometry and immunohistochemistry.

Key words: basal cell carcinoma, histopathology, citokeratines, smooth muscle actine, proliferation index, apoptotic index.
I. STAGE OF KNOWLEDGE

Chapter I - "Skin morphophysiology" describes classical embryology and anatomy elements of the cutaneous organ, monitoring as separate entities the epiderm, the basal membrane, the derma, hypoderm and cutaneous annexes.

Chapter II with the title "Tumors -histopathology" is an introduction to the morphopathology of human tumors, then concentrating on epidermal tumors in general. After a brief description of the clonality of tumor cells, there is a review of the morphological characteristics differentiating the malign from the benign tumors, the criteria for classification of malignant tumors and the importance of the combined study on the stroma and tisular parenchyma. The chapter focuses on the characteristics of tumors with cutaneous localization.

Chapter III entitled "Basal cell carcinoma" describes the general data related to epidemiology, pathogenesis, clinic and morphopathology of basal cell carcinoma (BCC) in general.

Basal cell carcinomas (BCC) is situated in 90% of the cases at the level of cephalic extremity, especially at face level. With the exception of the nevoid basal cell carcinoma syndrome, they rarely appear on palms or on plants. Basal cell carcinoma usually appears as single lesions, although several lesions appearing simultaneously or consecutively should not be neglected.

Even though basal cell carcinoma may appear with a specific reasons, numerous factors involved in the appearance of basal cell carcinoma were described. The most common of them is white skin (phototype I or II) in association with prolonged sun exposure. Usually basal cell carcinoma don’t metastize. Still there are also exception. The incidence of metastasis varies from 0,01% in pathological tests, up to
0.028% in patients with dermatological diseases and 0.1% in patients from surgical centers.

Basal cell carcinomas share common characteristics of the cells in the epidermal basal layer: palisading of peripheral cell nuclei, a specialized stroma and cleft-type artifacts in the junction between epithelium and stroma. Besides, there are various degrees of citologic atypia and mitotic activity; and this is almost always present in certain level of these last two modifications.

From the histological point of view basal cell carcinoma can be split in two large groups: non-differentiated (solid) and differentiated.

Cancerous tissue has two components: cancer cells and stroma. In BCC, the conjunctive stroma proliferates together with the tumor and is disposed in parallel fascicles around tumor masses, so that there seems to be a mutual relationship between the tumor parenchyma and its stroma. There are frequent areas of stroma retraction from the tumor islands, which leads to forming of peri-tumor lacunae.

From the imuno-histochemical point of view, the cells in the basal cell carcinoma islands are imuno-positive for keratine (especially keratins with low molecular mass), and usually negative for epithelial membrane antigen (EMA), carcino embryonic antigen (CEA), and involucrin. Also MAK-6 antibody (another pacito-keratine) is negative in BCC.

The basal membrane surrounding the tumoral nests reacts to anti-laminin antibodies, colagen IV, colagen V and bulous pemphigoid antigen. Reactivity for these antibodies tends to vary in intensity and in continuity in the more aggressive forms such as morpheiform and infiltrative BCC.
II. OWN CONTRIBUTIONS

The current study approached the following aspects:


- Epidemiological characteristics of BCC cases in the analyzed region and period

- Analysis of morphological particularities depending on clinical form.

- Correlations between main markers used in the the imuno-histochemical description of BCC

- Can antigens with a prognosis role in the tumor evolution and extension be identified from these markers

Chapter IV entitled “Materials and methods” describes the study materials, as well as used techniques. Our basic materials is represented by the totality of cases reported as neoplasia of the skin by the Valcea County Hospital in the period 1999-2007 (Table 1).

<table>
<thead>
<tr>
<th>Year</th>
<th>Basal cell Carcinoma</th>
<th>Squamous cell Carcinoma</th>
<th>Malignant Melanoma</th>
<th>Kaposi disease</th>
<th>Precancerous lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>15</td>
<td>5</td>
<td>0</td>
<td>6</td>
<td>141</td>
</tr>
<tr>
<td>2000</td>
<td>26</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>144</td>
</tr>
<tr>
<td>2001</td>
<td>24</td>
<td>7</td>
<td>5</td>
<td>1</td>
<td>250</td>
</tr>
<tr>
<td>2002</td>
<td>26</td>
<td>8</td>
<td>2</td>
<td>1</td>
<td>127</td>
</tr>
<tr>
<td>2003</td>
<td>25</td>
<td>14</td>
<td>1</td>
<td>0</td>
<td>132</td>
</tr>
<tr>
<td>2004</td>
<td>34</td>
<td>10</td>
<td>1</td>
<td>0</td>
<td>119</td>
</tr>
<tr>
<td>2005</td>
<td>20</td>
<td>15</td>
<td>3</td>
<td>5</td>
<td>143</td>
</tr>
<tr>
<td>2006</td>
<td>44</td>
<td>20</td>
<td>0</td>
<td>4</td>
<td>138</td>
</tr>
<tr>
<td>2007</td>
<td>39</td>
<td>17</td>
<td>3</td>
<td>3</td>
<td>102</td>
</tr>
<tr>
<td>Total</td>
<td>253</td>
<td>100</td>
<td>16</td>
<td>24</td>
<td>1296</td>
</tr>
</tbody>
</table>

Table 1. Spectrum of dermatological pathology in Vâlcea county for the period 1999-2007.
From the 253 cases subsequently confirmed as basal cell carcinoma, a randomized selection of 50 cases was done to the imuno-histochemical study (Table 2).

<table>
<thead>
<tr>
<th>Histopathological form</th>
<th>No. of Patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solid</td>
<td>25</td>
<td>50.00%</td>
</tr>
<tr>
<td>Morpheiform</td>
<td>9</td>
<td>18.00%</td>
</tr>
<tr>
<td>Infiltrative</td>
<td>8</td>
<td>16.00%</td>
</tr>
<tr>
<td>Adenoid</td>
<td>6</td>
<td>12.00%</td>
</tr>
<tr>
<td>Superficial</td>
<td>2</td>
<td>4.00%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2.** Histopathological subtypes of BCC from the study lot

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Epitope / marker</th>
<th>Dilution</th>
<th>Antigen retrieval</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smooth muscle actine</td>
<td>Muscle actine, miofibroblasts</td>
<td>1:100</td>
<td>Citrate, pH6</td>
<td>Dako</td>
</tr>
<tr>
<td>Bcl-2 Bcl-2</td>
<td>Oncoproteine bcl-2</td>
<td>1:100</td>
<td>EDTA, pH9</td>
<td>Dako</td>
</tr>
<tr>
<td>Activated caspase 3</td>
<td>Apoptotic Marker</td>
<td>1:101</td>
<td>Citrate, pH6</td>
<td>Cell Signaling</td>
</tr>
<tr>
<td>CD20cy CD20cy</td>
<td>Lymphocytes B</td>
<td>1:100</td>
<td>Citrate, pH6</td>
<td>Dako</td>
</tr>
<tr>
<td>CD3 CD3</td>
<td>Lymphocytes T</td>
<td>1:100</td>
<td>Citrate, pH6</td>
<td>Dako</td>
</tr>
<tr>
<td>CD31 CD31</td>
<td>Vascular endothelium</td>
<td>1:100</td>
<td>Citrate, pH6</td>
<td>Dako</td>
</tr>
<tr>
<td>CD34 CD34</td>
<td>Vascular endothelium</td>
<td>1:100</td>
<td>Citrate, pH6</td>
<td>Dako</td>
</tr>
<tr>
<td>CD68 CD68</td>
<td>Macrophages/monocytes</td>
<td>1:100</td>
<td>Citrate, pH6</td>
<td>Dako</td>
</tr>
<tr>
<td>Citokeratine 19</td>
<td>Citokeratine with low molecular mass</td>
<td>1:100</td>
<td>Citrate, pH6</td>
<td>Abcam</td>
</tr>
<tr>
<td>Citokeratine 34βE12</td>
<td>Citokeratine with high molecular mass</td>
<td>1:100</td>
<td>EDTA, pH9</td>
<td>Dako</td>
</tr>
<tr>
<td>ki-67 ki-67</td>
<td>Proliferation factor</td>
<td>1:50</td>
<td>EDTA, pH9</td>
<td>Dako</td>
</tr>
<tr>
<td>Laminin Laminin</td>
<td>Basal membranes</td>
<td>1:50</td>
<td>Proteinase K</td>
<td>Dako</td>
</tr>
</tbody>
</table>

**Table 3.** Antibodies used in this study
From the pieces included for paraffin seriated sections were cut with a thickness of 4 μm. Histochemically, **classical staining** procedures were used, such as haematoxylin-eosin, light green according to Goldner-Székely method and PAS (Acid Periodic Schiff).

**For the imuno-histochemical study**, the same biological material was used as for the histological investigations. The imuno-histochemical technique per se included a standard algorithm, with some variations depending on the used antibodies (Table 3).

**The two dimensional automated morphometry** was aimed at:

- Semi-quantitative measurement of the density in the collagen fibers
- Quantification of inflammatory infiltrate and comparison between different pathological variants
- Calculation of cell proliferation indexes
- Quantification of aria occupied by miofibroblasts

**Three dimensional morphometry.** The use of sets of 50 sections imuno-marked for the blood vessels resulted in a high resolution three dimensional reconstruction of the epithelium and vessels in the surrounding conjunctive atmosphere. This allowed the measurement in all three axes of the distances between vessels and epithelial cells, as well as the volumetric measurement of the epithelium and underlying conjunctive tissue.

**Chapter IV - “Results”** presents the main results of the paper in 5 sections, as follows:

1. **Epidemiological study** showing the evolution of BCC incidence

2. **Clinical study** showing that **nodular BCC** was the most frequent form described in the studied group, with most
frequent localization at the face (eyelids, nose) and throat, according to areas with highest sun exposure.

3. **Histopathological study** proved that solid BCC represented the most frequent histological form identified on the pieces included in the study, clinically adequate to the pearl-like nodular or nodal-ulcerated forms. This is characterized by the presence of smaller or larger bundles of basaloid cells situated at the level of papillary or reticular derm, accompanied by characteristic stromal retractions (clefts) (Fig. 1).

![Figure 1. Solid BCC – presence of bundles of separate basaloid cells. Col. HE, x 40](image)

Also the density of the stroma (and implicitly of the stromal cell component) was higher towards the reticulated derm compared with papillary derm (the apical part of the basaloid proliferation), indicating a desmoplastic reaction proportional with the initial density in the surrounding conjunctive tissue.

In the decreasing order of frequency, the following were the cases classified as morpheiform and adenoid BCC. The presence of cells in mitosis and even atypical mitosis and frequent apoptotic bodies was recorded in infiltrative forms.
4. **The imuno-histochemical study** focused on the analysis of the markers listed in the chapter „Materials and Methods”

4.a. The expression citokeratines with high molecular mass showed a strong reactivity in general for all pathological forms studied.

4.b. The study continued with the histological analysis of the proliferative and apoptotic activity through immunomarking for Ki-67 and activated caspase 3.

![Proliferative and apoptotic indexes in BCC](image)

**Figure 2.** Apoptotic and proliferative index in BCC.

The comparison of the proliferative with the apoptotic index showed constantly higher values for the latter compared with the proliferative index (Fig. 2).

4.c. **Examination of lymphocyte infiltration** showed a B type lymphocyte infiltrate maximal in morpheiform BCC stroma, compared with the adenoid and solid forms. The semi-quantitative morphometric analysis showed a T type type lymphocyte infiltrate maximal for the peritumoral stroma of morpheiform BCC, compared with adenoid and solid forms.
4.d. The study on the expression of smooth muscle actine showed variable intensities, proportional with a more severe histopathological type of the disease.

4.e. Studying the bcl-2 expression I could appreciated that the morpheiform BCC had the maximum marking intensity followed by adenoid and solid BCC forms.

5. The morphometric study identified a decrease in the fibrosis degree from the morpheiform BCC to the adenoid and solid form. The results also showed an increase in the stromal nuclear density, from the solid form to the adenoid, respectively morpheiform.

**Figure 3.** Vascular density in BCC.

Analysis of the blood vessels distribution in the peritumoral stroma showed that within a 30-60 μm distance of the epithelioid cells, the number of vessels was to a maximum, while levels were lower according to distance or very close to the tumor (Fig. 3).

Three dimensional analysis of the relationship of basaloid cells with suprajacent epithelium revealed permanent
contact of the tumor nests with epidermal basal layer, irrespective of the studied form (Figure 4).

Figure 4. Seriate sections and 3D reconstruction of adenoid BCC. Vessels do not appear intra-tumoral in the conjunctive tissue.

Conclusions

1. The epidemiological profile of the patient with CBC in Valcea County between 1999-2007 was: male aged between 60-80; urban environment; located in the face; predominant clinical form: nodular ulcerated; dominant morphological form: solid nodular

2. The virulence of the tumor decreases from infiltrative type, to morpheiform, adenoid and then solid. This was correlated and resembles to the pattern of other literature date published.

3. This study shows a variable expression of cytokeratins in CBC. Their expression cannot fit thus within a set pattern not even within the same morphological forms.

4. The apoptotic index is higher to the proliferation index, and both have higher values in more infiltrative forms. This seems to be a key feature limiting the infiltrative aspect of the tumor
and increases proportionally in aggressive forms thus offsetting the more aggressive cellular phenotype.

5. The SMA expression and bcl-2 correlate with CBC aggressive forms, a fact confirmed by such recent literature data.

6. The need for permanent contact with the epidermal base layer or the external theca of pillous follicle may bring in the future details on the necessity for a possible micro-environment incompatible with metastasis in such tumors.

7. In general the vascular density is increased around the tumor, but not in direct contact with it. In the future it would be interesting to assess what molecular signs block vascular closeness to the tumor.

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**PUBLICATIONS**

**Presented papers**


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Immunohistochemical nuclear staining for p53, PCNA, Ki-67 and bcl-2 in different histologic variants of basal cell carcinoma. Mateoiu, C., Pirici, A., Bogdan, F. Rom J Morphol Embryol. 52(1 Suppl):315-9319; 2011 – Journal indexed ISI, FI=0.4

