DOCTORAL THESIS

CONTRIBUTIONS TO THE STUDY OF BASAL CELL CARCINOMA.
HISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY

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CRAIOVA
2011
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INTRODUCTION

The objective of this work was to contribute to understanding the clinical behavior and histopathology of basal cell carcinoma, mainly based on cases encountered in sections of Dermato and plastic surgery of the County Emergency Hospital of Craiova. We were motivated by finding increased incidence of basal cell carcinoma in recent years.

In this paper we tried to evaluate the existing mutual morpho-functional correlations both in normal and lesional skin with clinical and prognostic aspects in order to better understanding the development and evolution of basal cell carcinomas. The characteristics of neoplastic process were studied by a clinical study, a histological and immunohistochemical one.

GENERAL PART

CHAPTER I
Morphophysiology of the cutaneous tissue

The skin accounts for about 15% of the total body weight and is the largest organ of the body. It is composed of three layers: (a) epidermis, (b) dermis and (c) the subcutaneous adipose tissue. Each component has its unique and complex structure and function, with variation according to age, gender, race, and anatomic location. Functions of the skin are extremely diverse. It serves as a mechanical barrier against external physical, chemical, and biological noxious substances and as an immunologic organ. It participates in body temperature and electrolyte regulation. It is an important organ of sensuality and psychological well-being. In addition, it is a vehicle that expresses not only primary diseases of the skin, but also diseases of the internal organs. An understanding of the skin's normal histology is essential to the understanding of pathologic conditions [1].

Embryologically, the ectoderm gives rise to epidermis and its appendages. The mesoderm provides the mesenchymal elements of the dermis and subcutaneous fat [2].

Most epithelial cells of skin appendages derive from follicular epithelial stem cells localized in the basal layer of epidermis at the prominent bulge region of the developing human fetal hair follicles. Furthermore, such multipotent stem cells may represent the ultimate epidermal stem cell [3].
CHAPTER II

Skin carcinogenesis

2.1. Etiological factors involved in skin carcinogenesis

Findings regarding the genetic basis of non-melanoma skin cancer (NMSC) have confirmed that UV radiation, especially UVB (290-320 nm in the solar spectrum), contributes to the formation of squamous and basal cell carcinomas [4].

NMSCs are caused by genetic abnormalities, most often induced by UVB exposure. Actinic keratoses, which lead to SCCs, have gene mutations in Kras, H-rasV12 and cyclin dependent kinase 4 (CDK4) produce human epidermal neoplasia. Therefore, a combination of these genetic abnormalities might be crucial to the carcinogenesis at least in a subset of SCCs. High doses of ultraviolet light can also lead to skin cancers by inducing reactive oxygen species (ROS) that play an important role in tissue injury. Increased production of ROS and/or decreased efficiency of antioxidant defence system contribute to a number of degenerative processes including cancer. Common exogenous carcinogenic agents in addition to UV radiation include 1) tobacco use, 2) human papilloma viruses, 3) arsenic, 4)industrial chemicals such as vinyl chloride, polycyclic aromatic hydrocarbons, 5) MNNG (N-methyl-N’-nitro-N'-nitrosoguanidine), an alkylating agent, and 6) exposure to gasoline or gasoline vapours [5].

2.2. Alterations in cell physiology that collectively dictate malignant growth

According to Douglas Hanahan şi Robert Weinberg [6] the vast catalog of cancer cell genotypes is a manifestation of six essential alterations in cell physiology that collectively dictate malignant growth: self-sufficiency in growth signals, insensitivity to growth-inhibitory (antigrowth) signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis. Each of these physiologic changes—novel capabilities acquired during tumor development—represents the successful breaching of an anticancer defense mechanism hardwired into cells and tissues. We propose that these six capabilities are shared in common by most and perhaps all types of human tumors. This multiplicity of defenses may explain why cancer is relatively rare during an average human lifetime. We describe each capability in turn below, illustrate with a few examples its functional importance, and indicate strategies by which it is acquired in human cancers.

2.3. Cutaneous tumors classification

Pentru clasificarea tumorilor epidermului de suprafaţă s-a folosit clasificarea dată de către Organizaţia Mondială a Sănătăţii (WHO) [7].
CHAPTER III

Basal cell carcinoma

Basal cell carcinoma represent a group of malignant cutaneous tumours characterised by the presence of lobules, columns, bands or cords of basaloid cells ("germinative cells") [8].

3.1. Epidemiology

The incidence of skin cancer has markedly increased over the past few decades. At this time, between 2 and 3 million nonmelanoma skin cancers (NMSCs) and approximately 132,000 melanoma skin cancers occur globally each year [9].

3.2. Pathogenesis

BCC is the indolent malignant neoplasm of the hair follicle and emerges from keratinocyte stem cells in hair follicles, sebaceous glands, or interfollicular basal cells [10,11].

3.3. Diagnosis

Nodular/noduloulcerative BCCs are the most common. Look for pearly, waxy papules or nodules with raised or rolled borders and central small ulcers. The multiple variants of basal cell carcinoma (superficial, morpheaform, basosquamous, pigmented, infiltrative, giant) are connected by the common histological feature of lobules, columns, bands and cords of basaloid cells ("germinative cells") associated with scant cytoplasm and a characteristic outer palisade of cells associated with a surrounding loose fibromucinous stroma. Artefactual retraction spaces between the tumour and stroma are often present. The tumour-stromal interaction is weakened by the characteristic lack of the hemidesmosomes that anchor the normal epidermis to the dermis. Apoptosis is usually apparent. The release of keratin into the stroma as a result of apoptosis may lead to the formation of amyloid deposits [12,13].

3.4. Prognosis and predictive factors

Basal cell carcinomas are locally invasive tumours and metastases occur in less than 1 in 10,000 tumours. Morbidity is increased with deeply invasive tumours and with neglected tumours that may measure more than 10 cm in diameter, which may extend into the deep tissue to bone and follow fusion planes particularly on the face where they follow nerves through bony channels. Increased recurrences are associated with infiltrative, morphoeic and micronodular basal cell carcinomas as surgical margins may be underestimated. Distance to the closest resection margin is an important predictor of BCC recurrence [14].

3.5. Treatment

Currently, these therapeutic methods are used: surgical excision, one of the most common treatments, curettage and electrodesiccation, cryosurgery, radiation therapy, Mohs’ micrographic surgery, laser surgery and photodynamic therapy, interferon, imiquimod, 5-fluorouracil, chemoprevention, such as with retinoids and cyclooxygenase inhibitors [15].
SPECIAL PART

CHAPTER IV
Clinical study

1. Distribution of the cases raported to age, sex and area of origin of the patients

   Over the years, frequency of BCC is irregular and the number of diagnosed cases has increased in recent years (from 122 in 2003 to 197 in 2009). Of 1072 cases studied, 497 (46.36%) were male patients and 575 (53.64%) were female. Sex ratio is 1.15 for women. In this study BCC occurrence was observed in patients aged 17 years and 94 years, with a prevalence between 55-73 years (55.4%), and the average age was 55.5 years.

   BCC patients repartition of the study group according to age groups shows that the BCC has a maximum frequency in the age group 55-73 years, which affects 594 patients (55.4%) and in the age group 17-35 years, the number of patients is very low (14 patients - 1.3%).

   Maximum difference between the sexes is found in the age group 55-73 years, where BCC was found in 357 men (60.1%) and 237 women (39.89%). 398 cases came from urban areas (37.13%) and 674 cases (62.87%) from rural areas.

4.2.2. Distribution of the cases raported to appearance of lesions

   After surgical ablation in 978 patients were resulted 1072 tumors, because some of them had multiple tumor lesions with different locations. Results show that in the 1072 BCC cases, 974 cases (90.85%) are located in the head and neck, 85 cases (7.93%) in the trunk and limbs, and in 13 cases (1.21%) location was not specified.

   The presence of the head was found in 961 cases (89.64%), and the neck 13 cases (1.21%). Most cases of BCC were found in the nose (337 cases) and the fewest in the occipital region (7 cases). 34.60% of cases were found in the nasal region, which was followed by genian region (15.40%) and orbital region with 11.60%. Ear, frontal, temporal and infraorbital regions are areas in which the frequency varies quite low, respectively between 8.52% in the ear region and 6.06% in the temporal region.

   Distribution of cases according to clinical appearance of BCC showed that the largest share was held by nodular BCC - 524 cases (48.88%), followed by ulcerated form - 118 cases (16.98%). The last places are occupied by vegetative form and morphea-like (with 9 respectively 6 cases - 0.86% and 0.65% of all studied cases).
CHAPTER V
Histological study

Among the features described in the literature, we found the following histopathological types of CBC in our study:

<table>
<thead>
<tr>
<th>Histopathological type</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>nodular</td>
<td>471</td>
</tr>
<tr>
<td>adenoid</td>
<td>213</td>
</tr>
<tr>
<td>keratotic</td>
<td>97</td>
</tr>
<tr>
<td>pigmented</td>
<td>89</td>
</tr>
<tr>
<td>metatypical</td>
<td>73</td>
</tr>
<tr>
<td>superficial</td>
<td>56</td>
</tr>
<tr>
<td>cystic</td>
<td>32</td>
</tr>
<tr>
<td>infiltrative</td>
<td>23</td>
</tr>
<tr>
<td>morphea-like</td>
<td>18</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>1072</strong></td>
</tr>
</tbody>
</table>

Table 1. Distribution of cases by histopathological type

The present study showed invasion in 66/1072 cases - 5.83% of all tumor lesions analyzed. Of histopathological subtypes included in this study, infiltrative BCC had presented invasion most often: 19/23 cases - 82.60% and less, pigmented BCC: 2/89 cases - 0.22%.

The degree of depth invasion meant infiltration in dermis, the skin appendages, skeletal muscle fibers, adipose tissue, cartilage and perineural invasion.

The distribution of invasive BCC depending on the degree of invasion shows that most invasive BCC invaded deep to the hypodermis (31/66 cases - 46, 97%), followed by those who have invaded up to the striated muscle fibers (17/66 cases - 25.76%) and only 6.06% (4/66 cases) invaded only by dermis. Of the 1072 cases of BCC, only 37 were relapses (3.41%) and in 93 cases (8.68%) diagnosis on hospitalization was inconsistent with histological diagnosis.

In this study, the analysis of 1072 tumor lesions from the 978 patients on standard stains: hematoxylin-eosin, tricomic Masson, van Gieson and special stain: PAS and orcein stain included the next histopathological types: solid (471/1072 cases 43.94%), adenoid (213/1072 cases, 19.87%), keratotic (9.05%), pigmented (89/1072 cases, 8.39%), metatypical (6.81%, which was observed in 73 cases), superficial (56/1072 cases, 5.22%), cystic (32/1072 cases, 2.99%), infiltrating (23/1072 cases, 2.15%) and morphea-like (18/1072 cases (1.68%).
CHAPTER VI

Immunohistochemical study

Immunohistochemical study aimed to investigate the characteristics of tumor cells, the features and composition of the stroma and the relationship between them. Aspects that we investigated had the following objectives:

1. Citokeratins expression analysis

The expression of citokeratins was studied in a number of 51 cases of BCC (nodular, superficial, infiltrating and morphea-like subtypes), and determined using polyclonal antibodies Ber-EP4 (17 cases), 34βE12 (21 cases), AE1/AE3 (13 cases) and EMA (32 cases).

2. Proliferation factor analysis

Although all types of BCC have some common histopathological aspects, there are the defining features for each variant. The present study included 41 cases of BCC. Immunohistochemical stain for characterization of cell proliferation were performed for PCNA, p53, Ki67 and Bcl-2, and histopathologic subtypes included in the study, classified by predominant histological pattern were: superficial (8 cases), nodular (13 cases), infiltrating (9 cases) and sclerosing (11 cases) BCC.

3. Peri- and intratumoral stromal elements analysis

This study involved the evaluation of immune response of tumor and stromal cells to immunostain with vimentină, α smooth muscle actin (αSMA), collagen IV, CD34, CD105 and CD44. Actually, all of them are antibodies currently used for the control of the multiplication and differentiation mesenchymal elements.

4. Inflammatory element analysis

We studied the expression of T lymphocytes on a number of 32 cases of BCC, each 8 cases of each subtype included in the study, using antibodies: Foxp3, IL 17, UCHL1, CD4 and CD8. Immunohistochemical evaluation of B lymphocytes was performed on a total of 32 cases, of which 27 were inconclusive, and of these only 19 were positive. In 7 of the 19 positive cases had available nodular character, and the remaining 12 was dispersed, diffuse. Response to L26 has mainly moderate intensity (4 cases) and low (13 cases) and only 4 cases highly positive. Distribution of dendritic cells in BCC has been investigated using marker S100. Intensity was weak positive reaction in 11 cases (40.74%) and moderately positive in 8 cases (29.62%). Five cases were negative for S100 and three cases were intensely positive. Response to CD117 intensity was moderate in 13 cases and poor in 16 cases. Three cases had a strong positive reaction and one case was negative.
CHAPTER VII
General discussions

Basal cell carcinoma is the most common form of skin cancer, accounting for approximately 80% of malignant skin tumors. It seems that the incidence has increased by about 10% per year.

It is believed that the BCC has its origins in pluripotent cells of the basal layer of epidermis. Perhaps as a consequence of its origin, there is considerable variation among histopathological CBC, which allowed description of several subtypes. The most common histological subtypes are nodular and superficial.

Risk factors for development of BCC include exposure to ultraviolet radiation and other skin diseases and immunosuppression. It usually occurs on sun-exposed areas of skin, most often in the head and neck, followed by trunk, and extremities. Aftab M et al [16] presented a case with conjunctival location.

Clinical appearance is quite variable but usually lesions are described as papules or nodules, pearl, glowing with a limit of demarcation can be represented by the crust, ulceration or bleeding. Five clinical types of basal cell carcinoma occur: noduloulcerative basal cell carcinoma, including rodent ulcer, by far the most common type; pigmented basal cell carcinoma; morphea-like or fibrosing basal cell carcinoma; superficial basal cell carcinoma; and fibroepithelioma. Significant correlations between BCC subtypes and stromal and immune alterations were found: superficial BCC correlated with late recurrence and moderate to dense lymphocytic inflammatory infiltrate, high-risk subtypes were correlated with active recurrence; infiltrative and morpheiform variants - with stroma dense fibrous [17].

There are some hints on BCC control by the immune system. Curson and Weedon described CBC infiltrating immune cells belonging to possible signs of regression (disruptive architecture of tumor cells located in the palisade at the periphery of the tumor, the appearance of apoptotic cells and dermal collagen deposits). Based on these criteria they found that 81 out of 400 examined tumor regression [18].

Recently, Finn and Forni revealed that the reason of the preventive immunization against malignancies is strong [19]. When the vaccine held before cancer development, the host immune system was not affected by tumor-induced suppression, and it can remove tumors more effectively than clinically silent on the macroscopic observables. Indeed, recent studies in animals have shown that cancer vaccines are not only capable of protecting against tumor development, but may reduce tumors in genetically predisposed animals. There are tumors that manage to escape the immune response, an aspect that remains to be clarified.
CHAPTER VIII

Conclusions

1. Basal cell carcinoma occurs with greater frequency in recent years. Its incidence is increased in women. The age group showing the highest number of cases is from 55 to 72 years - which entitles us to consider it preserve body skin senescence, but it is worth noting an increase of its occurrence in the young population.

2. In the studied group, which amounted 1106 patients, carriers of the 1072 tumors, 398 (37.13%) came from urban areas and 674 (62.87%) from rural areas, 978 (91.23%) cases were located on sunlight exposed areas (head and neck), the nose and cheek region, and rarely on the trunk and limbs.

3. Histological tumor behavior was similar regardless of location or mode of sampling (surgical excision or biopsy tumor).

4. The study highlighted the importance of skin malignant pathology both by the large number of cases studied and the rising incidence of basal cell carcinomas.

5. Immunohistochemical study of citokeratins confirmed the epidermal origin of cells involved in BCC. The citokeratins expression heterogeneity is demonstrated by their immunofenotipical variability. BerEP4 remains a useful and reliable marker for the BCC and it was confirmed in all 17 cases studied, except for one case, but EMA one marker particularly useful in the differential diagnosis with squamous and bazoscuamos cell carcinoma was negative in 30/32 cases being studied.

6. Positivity to vimentin may also be an indicator of epidermal origin of the BCC, the α-actin positivity has role in the assessment of aggressive and invasive feature of BCC; the α-actin tumor stroma positivity suggests the possibility of miofibroblastic stromal changes; peritumoral expression level of collagen IV reflects an alteration of the interaction of the neoplastic epithelium with its conjunctive stroma and suggests a more aggressive BCC of those cases; CD34 and CD105 intensity response is also associated with tumor aggressiveness; CD44 expression was low in all histological subtypes studied and this aspect is considered one of the factors that block the formation of metastases in BCC.

7. The analysis of proliferation factors showed that Bcl-2 and p53 had a strong tendency to indicate the severity of BCC, which might suggest that BCC slow progress, relatively benign, was due to increased apoptosis, and Ki67, due to its variable behavior can not be considered a marker of severity, also PCNA was not a good marker of cell proliferation.

8. The inflammatory infiltrate study finds utility in immunization with specific activated proteins by hedgehog signaling pathway which may hold the promise of preventive options for patients with basal cell nevus syndrome prone to develop a large number of BCC. For these patients and others who develop multiple BCC, even a partial reduction of tumors could substantially improve their quality of life.

9. Increased incidence of non-melanomatous skin cancer, and hence the economic costs to society, in addition with an aging population emphasizes the need of prevention and less expensive treatment.

10. New therapeutic methods are necessary due to recurrence, multiple tumors, complicated methods of treatment, cosmetic results and local adverse reactions. Blocking hedgehog signaling pathway with GANT compounds appears to be a promising strategy, although it has not yet been clinically evaluated.

11. The most effective way to decrease the frequency of non-cutaneous malignancy in various forms is prevention, which aims at both reducing exposure to ultraviolet radiation and early detection.
BIBLIOGRAPHY

1. (1) Darke Richard, Wayne Vogl, Adam W. M. Mitchell - Gray’s Anatomy for students, Elsevier, 2005
2. (2) Stoicescu Irina - Patologie cutanată tumorală (note de curs), Ed. Sitech, Craiova, 2006
12. (65) David E Elder, ChB Rosalie Elenitsas, Bernett L Johnson Jr., George F Murphy, Xiaowei Xu - Lever's Histopathology of the Skin, Lippincott Williams & Wilkins, 2008
16. (109) Heckmann Marc, Zogelmeier Frank, Konz Birger - Frequency of Facial Basal Cell Carcinoma Does Not Correlate With Site-Specific UV Exposure