DOCTORAL THESIS

ABSTRACT

CARCINOMAS OF THE CEPHALIC EXTREMITY. HISTOLOGICAL, CLINICAL AND IMMUNOHISTOCHEMICAL STUDY

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Key words: basal cell carcinoma, risk factors, histopathological variations, immunohistochemical markers, skin
INTRODUCTION

Malignant tumours of the skin, because of their high morbidity and potential gravity, are a scientific interest field and an important medical and social problem. The high incidence is explained by many factors: sun exposure, change in dress code, longer life expectancy, and diminished ozone layer.

The high incidence of non-melanoma skin cancer can be reduced through smaller ultraviolet exposure and early detection (by skin screening). Treatment is complicated because of recurrence, multiple tumours, local side effects with skin defects and general side effects following radiotherapy and chemotherapy. There is often need for plastic reconstruction for esthetical purpose.

This paper is a warning signal for plastic surgeons and for other surgical specialities connected to head and neck pathology, towards one of the most difficult forms of skin cancer.

The purpose of this research was understanding clinical, histopathological and immunohistochemical behaviour of basal cell carcinoma of the head and neck, looking through the patients of the Plastic Surgery Clinic of the Craiova Emergency Clinical County Hospital. The motivation was given by the high incidence, morbidity, aggressiveness and recurrence, diagnosis difficulties, treatment difficulties, the different clinical forms it may have, and treatment options.

STATE OF KNOWLEDGE

CHAPTER 1
HISTOLOGY AND HISTOPHYSIOLOGY OF THE SKIN

The cutaneus organ represents the skin and skin annexes (sebaceous and sweat glands, hair, nails). It is the coating structure of human organisms and it has specific qualities adapted to its function [Darke Richard, Wayne Vogl, Adam WM Mitchell, 2005]. It represents a mixture of epithelium, connective, muscle, vascular and nervous tissue. [Toader Radu M, Drăgan AM, Pântea A, 2001].

Skin is made of an ectodermal superficial layer, the epidermis, a mesodermal layer of connective tissue, the dermis, and a deep layer of connective fat tissue, the hypodermis, which binds the skin to underlying structures.

The epidermis is a stratified malpighian squamous epithelium with keratinization undergoing a continuous process of renewal. It consists mainly of cells with limited life cycle time, keratinocytes, of ectoblastic origin, evolving towards keratinization. There are, in addition to keratinocytes, other cell types with different functions and origin, which represent 5-10% of the epidermal cells: melanocytes, immune cells (Langerhans cells, dendritic cells, Grandstein cells), Merkel cells. The epidermis presents five cell layers: stratum basale, stratum spinosum, stratum granulosum, stratum lucidum, the stratum corneum. On the surface, the stratum corneum cells peel, constituting the sixth layer described in classical histology, flaky layer.

The dermis has two areas: a superficial area, papillary dermis and a deep area, reticular dermis. Superficial dermis (papillary) consists of loose connective tissue well vascularised and innervated, and very rich in cells: fibroblasts, mast cells, macrophages. Deep dermis (reticular) consists of connective tissue rich in fibrillar elements. Of fibers, the collagen predominates.
Skin annexes are glandular structures (sebaceous glands and sweat glands) and appendages (hair, nails).

The skin has multiple important functions for the body. Also, because of the epidermal cells, that have a high capacity for proliferation, it can regenerate and heal. With age, however, like other tissues and organs, the skin undergoes an aging process.

CHAPTER 2
CLASSIFICATION OF SKIN TUMOURS

Unlike visceral cancers, skin cancers differ by: polymorphism, which hampers clinical diagnosis, making it essential for Histopathological examination; clear carcinoma predominance (about 2/3 of all skin cancers); in most cases the lesions evolve from precancerous lesions; important role, even decisive, of extrinsic factors in the emergence of these cancers, which explains their occurrence in uncovered areas in 90% of cases.

There are two types of tumors: benign and malignant tumors or cancers. The most significant difference between these types of tumors is that malignant ones have the ability to generate secondary or metastasized tumors. Between malignant and benign tumors there are clear criteria for differentiation. Thus, benign tumors are well delineated, encapsulated, slow growing, do not invade surrounding tissues, rarely metastasize, do not reappear after excision, are similar histologically to the tissue of origin, and mitoses are very rare. They are proliferating tumor formations with normal cells derived from skin cells [Forsea D, Popescu R, Popescu CM, 1998]. Unlike them, malignant tumors are badly delimited, fast-growing, invade surrounding structures, presenting possible relapses after excision, local and remote metastasis, are less similar to the tissue of origin, provide frequent mitoses and invade nearby satellites. Malignant skin tumors represent 15-20% of all cancers [Le Boit PE, Burg G, Weedon D, Sarasin A, 2006]. Their classification is made after their histogenesis.

CHAPTER 3
BASAL CELL CARCINOMA ETIOPATHOGENESIS AND EPIDEMIOLOGY

Skin cancer is much more common than cancer in the location of another organ or tissue, representing 4/5 of total cancers. This title consists of three anatomical clinical forms: basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and malignant melanoma (MM). BCC and SCC, named non-melanoma skin cancers (NMSC), are the most common forms of skin cancers, representing approximately 96% [Armstrong BK, Kricker A, 2001; Anthony ML, 2000]. Mucous membranes are usually not affected, but it may extend to them from the skin. The most frequent location of BCC is the head and neck (90%) and especially the face [Sartore L, Lancerotto L, Salmaso M, Giatsidis G, Paccagnella O, et al, 2011]. It is estimated that one in five Americans will develop skin cancer during their life, due to the decrease in the ozone layer and increased recreational sun exposure.
Birch-Johansen F, Jensen A, Mortensen L, Olesen AB, Kaer SK. (2010), have shown that the incidence of NMSC in Germany and Denmark is approximately the same (121/100,000 inhabitants in 2007). In Wales, the study carried out by Holme SA, Malinovsky K, Roberts DL (2001) showed an incidence of 104 cases in men and 83 women in 100,000 inhabitants.

Occurrence and development of skin cancers are determined and influenced primarily by extrinsic factors, whose intervention is more obvious than in the case of internal cancers and then by intrinsic factors, some of them common with those of internal cancers. Epidemiological data demonstrates that there is a direct relationship between the incidence of NMSC and exposure to the sun radiation especially UV. Repeated exposure to ultraviolet B (UVB 280-320nm) is known to be an inducer of BCC and SCC. It was proven that people who work outside and are chronically exposed to solar radiation, have a 43% higher risk of developing BCC and 77% for developing SCC [Bauer A, Diepgen TL, Schmitt J 2011; Schmitt J, Seidler A, Diepgen TL, Bauer A, 2011]. Currently there are scientific evidence supporting this view, the fact that UV radiation are important etiological factor in inducing cutaneous malignant melanoma, basal cell carcinoma and squamous cell carcinoma [Wang SQ, Setlow R, Berwick M, Polsky D, Marghoob AA, Kopf AW et al, 2001].

**PERSONAL CONTRIBUTIONS**

**CHAPTER IV
CLINICAL AND STATISTICAL STUDY**

We carried out a retrospective study on 349 patients hospitalized in the Plastic Surgery Clinic of the County Emergency Clinical Hospital Craiova in the period 2011-2013, who presented the diagnosis of Basal Cell Carcinoma (BCC) of the cephalic extremity.

Literature data indicate the development of basal cell carcinoma with predilection to areas of skin exposed to the sun, about 80% of tumors developing in the face (30% in the nose) and neck [Chinem &, Miot, 2011; Kopke & Schmidt, 2002; Hoban et al., 2002]. However, cases have been reported in the genital regions, mammary areas, axils, abdominal, scalp, interdigital areas. [Avci et al., 2008; Benamar et al., 2005; Betti et al., 1997; Bordel et al., 2006; Montagliani et al., 2004; Pagliani et al., 1995; Redondo Martínez et al., 2000; Sarfati et al., 2010].

CBC with facial location developed in the second decade of life until the 10th decade, the highest incidence representing the seventh decade in which 23.21% of the total number of patients were included, and the average age of the studied cases was 62.66 years for women and 60.71 years for men. There was a slight prevalence of cases in males (1: 1.14). The most frequent topographical region affected by the basal cell carcinoma was the nose (27.51 %) followed by the cheeks (20.34%). Among the regions which are less affected by the proliferation of carcinoma were in lower lip with 4.58% of cases and the chin with 6.59% region.
CHAPTER 5
HISTOPATHOLOGICAL STUDY OF BASAL CELL CARCINOMA OF THE FACE

The pathology of material derived from case studies of pathological anatomy laboratory of the Emergency Clinical Hospital Craiova and was represented by the archived paraffin blocks. These came from the histopathological processing of resection parts coming from patients operated on in the Plastic Surgery Clinic of the same hospital. The blocks and histological
diagnosis of clinical observation sheets were properly sorted out in the course of clinical and epidemiological research. Diagnostic strips have been reassessed in the light of the WHO criteria of diagnosis of keratinocyte tumors of the skin [Kossard et al., 2006].

Finally, for the time period investigated, 2011-2013, a total of 65 cases of basal cell carcinoma with facial location were selected as the object of study.

The histopathological study has investigated the main microscopic morphological characteristics of basal cell carcinoma.

**Staining methods** used:

- Hematoxylin-Eosin (H.E.) for reassessment of diagnosis according to the criteria of the WHO classification of tumours of the skin (Kossard et al., 2006);
- trichromic Masson staining with aniline blue for the degree of tumour fibrosis;
- Alcian blue-Periodic Acid Schiff (PAS-AA) in assessing the profile of goblet (neutral versus acidic) secreted by the tumour cells.

Histopathologically, the 65 cases investigated have shown a wide variety of morphological aspects, which are classified into the following sub-types: micronodular, shallow, nodular, morfeiform, cystic, adenoid, fibroepitelial, metatipic, keratotic, basal adnexal, pigmented and associated forms.

**Fig. 5.1** Superficial basal cell carcinoma—carcinoma island bounded peripherally by a cancer cell line. Col. H.E, X100

**Fig. 5.2** Micronodular basal cell carcinoma—micronodular aggregates with rare artifacts of stromal retraction. Col. H.E, X100
Typically, the morphological aspects characteristic for basal cell carcinoma regardless of subtype were: (1) the majority of carcinomatous cells showed basaloid morphology, (2) palisade aspect of nuclear cells of peripheral tumor and (3) the presence of artefacts between neoplastic proliferation and adjacent stroma.

Less than half of the cases (47.7%) were aggressive forms, invading adjacent dermis and hypodermis, and a percentage of 9, 23% showed perineural invasion.

Histopathologically, the most aggressive forms of facial basal cell carcinoma have been subtypes: morpheiphorm (100% invasive, 33% with perineural invasion), metatipic (100% invasive, 33% with perineural invasion), micronodular (100% invasive) and nodular (48.3%).
In addition, the stromal reaction of these tumors is sclerotic, basal cell carcinoma presenting a specific attraction less understood for connective tissue [Miller & Moresi, 2008].

Another particular aspect of such tumors is the attraction for perineural invasion, especially the deeply invasive specimens, literature data indicating an incidence of perineurale invasion between 0.18-3% [Walling et al., 2004].

CHAPTER 6
IMMUNOHISTOCHEMISTRY STUDY OF BASAL CELL CARCINOAMMA WITH FACIAL LOCALIZATION

Immunohistochemistry study using the material studied was represented by the 25 cases of facial basal cell carcinomas that have been the object of study of the histopathological exam.

The 25 cases were 15 female patients and 10 male patients aged 46 to 81 years (mean age was 64 years of age). Topographically, the cases have been diagnosed in the facial regions as follows: nasal region-9 cases; the skin of the lips-6 cases; periorbitar region 5 cases and 5 cases forehead region.

Histopathologically, the cases were placed in the following varieties of basal cell carcinoma: metatipic-6 cases; morpheiform-8 cases, micronodular 6 cases and 5 cases of superficial basal cell carcinoma. We must note that in relation to the initial case studies we have added 4 new cases of micronodular carcinoma and 3 new cases of metatipic carcinoma.

The antibodies used in this study were "targeting" mainly aggressiveness and prognosis of the facial basal cell carcinoamelor investigated. Thus, some were addressed to:

► tumour genesis: evaluation of CXCR4 and p53;
► local invasion appreciation in particular via: MMP-13, β-catenin, podoplanin and α-SMA, and by investigating the expression of p53 and CXCR4.

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**Fig. 6.1** Epidermis adjacent to carcinomatous proliferation. Citoplasmatic reactivity of spinous layer cells to CXCR4. Col IHC-DAB, X200.

**Fig. 6.2** hair follicle – Reactivity to CXCR4 Col IHC-DAB, X40

**Fig. 6.3** basal cell carcinoma morpheiform subtype– Reactivity for CXCR4 present in cytoplasm neoplastic cells. Col IHC-DAB, X100.
GENERAL CONCLUSIONS

- In the studied period, 2011-2013, facial basal cell carcinoma has accounted for about 88% of all basal cell carcinomas of the archives of the head and neck;
- Facial basal cell carcinoma was developed in the third decade of life until the 10th decade, maximum incidence cases being in the seventh decade (23.1%), and the average age of occurrence was 62.23 years;
- The report on gender has highlighted the prevalence of cases of male patients (1.1: 1), with an average age of facial basal cell carcinoma development lower to men compared to female patients (60.8 from 63.7 years);
The most frequent topographical region affected by the basal cell carcinoma was the nose (27.7%), followed by the location of the cheeks (20%) and, respectively, at the forehead (13.85%). At the same time, male patients tend to develop basal cell carcinomas, particularly in the periauricular region and on the chin, while in female patients these tumors have developed especially in the periorbitar and cheeks region.

A histological heterogeneity of the lesions was recorded, cases being investigated were placed in 11 subtypes (shallow, micronodular, nodular, morpheiform, cystic, adenoid, fibroepithelial, metatipic, keratotic, basal with adnexal differentiation and pigmented), and in four cases two such subtypes associations were present.

The most frequent histological subtype diagnosed in facial basal cell carcinoma is nodular type (44.6%), followed at some distance by morpheiform subtype (13.8%) and superficial type (10.7%).

Typically, the morphological aspects characteristic for basal cell carcinoma regardless of subtype were: (1) the majority of carcinomatous cells showed basaloid morphology, (2) peripheral palisade nuclear aspect of peripheral tumor cell and (3) the presence of artefactual clefts between neoplastic proliferation and adjacent stroma.

Less than half of the cases (47.7%) were aggressive forms invading adjacent hypodermis and dermis, and a percentage of 9.23% showed perineural invasion;

Histopathologically, the most aggressive forms of facial basal cell carcinoma have been subtypes: morpheiphorm (100% invasive, 33% with perineural invasion), metatipic (100% invasive, 33% with perineural invasion), micronodular (100% invasive) and nodular (48.3%).

The tegument fragments adjacent to tumor injuries we found reactivity for CXCR4, MMP-13 and β-catenin both in the epidermis (especially in its lower half) and cutaneous annexes, proving the involvement of these biomarkers in regenerative processes at this level.

At the edges of the tegument fragments of tumor resection of specimens investigated we noted the existence of "Epidermal clones" immunoreactive for p53, developed precisely in areas chronically exposed to the sun, which would play a major role in the development of skin cancers;

The high reactivity of the preneoplazic injuries associated biomarkers for type: CXCR4, MMP-13, β-catenine, p53 suggests their involvement in basal cell carcinogenicity;

Imunoreactivitaty of facial basal cell carcinoma to various investigated biomarkers has varied according to the histological subtype and lesion topography of these tumors.

Astfel, pentru biomarkerii de tipul CXCR4, MMP-13, β-catenină și D2-40, reactivitatea maximă am consemnat-o în varianta metatipică de carcinom bazocelular, îndeosebi la nivelul ariilor cu diferențiere scuamoasă. Cea mai slabă reactivitate am consemnat-o în variantele
micronodulare și superficial. Thus, for biomarkers type CXCR4, MMP-13, β-catenine and D2-40, maximum reactivity was recorded for the metatipic variant of basal cell carcinoma, particularly at the level of the areas with scuamous differentiation. The lowest reactivity was recorded for micro nodular and shallow.

In the case of p53, maximum reactivity was observed particularly in the micronodular variant of basal cell carcinoma, while subtype morpheiform has expressed the most intense α-SMA.

Regardless of the histological subtype of basal cell carcinoma, in all cases we investigated, the reactivity of the investigated markers for tumour was greater at the frontline of the invasion. In addition, at this level, the adjacent stroma showed an intense response for D2-40 and α-SMA, which is more obvious in the most aggressive forms of basal cell carcinoma in the metatipic subtype.

The reactivity for biomarkers of type CXCR4, MMP-13 and β-catenin was even higher for the endothelial cells and inflammatory cells in the inflammatory infiltrate associated to tumor framework. For α-SMA, stromal reactivity is more obvious in the vicinity of tumor invasion front;

We noted strong statistical correlations between scores of immunoactivity tumour biomarkers of CXCR4 and MMP-13, CXCR4 and β-catenin and MMP-13 and β-catenin while for p53 and α-SMA, the correlation was moderate, and one for p53 and D2-40 was weak;

All these results emphasize the need to investigate such biomarkers in order to identify the most aggressive forms of facial Basal Cell Carcinoma, offering the possibility of the development of more effective therapeutic strategies for such patients through direct targeting.

SELECTIVE BIBLIOGRAPHY


