UNIVERSITY OF MEDICINE AND PHARMACY CRAIOVA

DOCTORAL SCHOOL

PhD THESIS

The role of growth factors in progression of basal cell carcinomas

ABSTRACT

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SUMMARY

INTRODUCTION

Basal cell carcinoma is one of the most common malignant neoplasms, accounting for 70-80% of skin malignant tumors with high healing rates and excellent prognosis when it is diagnosed at early stages [53].

However, the aggression of basal cell carcinomas is variable and is dependent on some histopathological parameters represented by the subtype, size and tumor stage [7]. At the same time, although the basal cell carcinoma metastasis rate is low, the lesions show the local invasion capacity specific to malignant lesions.

Epidemiological data, frequency of lesions and associated risk factors, designate basal cell carcinomas as an important health problem worldwide.

The particular biological behavior of basal cell carcinomas in comparison with other malignant tumors and the absence of precursor lesions supported the research of biomolecular mechanisms underlying tumor initiation and progression as well as the prognostic role of histological differentiation for effective therapy [86,124].

In this context, the identification of biomarkers associated with the epidemiological and histopathological criteria of basal cell carcinomas aggressivity is a target of interest in determining the mechanisms involved in cutaneous carcinogenesis and can be a model of investigation of the lesions redarding the therapeutic management.

The study proposes the complex integrated epidemiological, histopathological and immunohistochemical investigation of the basocellular carcinomas in order to establish statistical relations, supporting the promotion of histological and molecular parameters as criteria for patients stratification for therapy.

KEYWORDS: basal cell carcinoma, histopathologic type, EGF receptors, KI67 proliferation index, TGFβ3/ TGFβRIII tandem

CHAPTER I. Epidemiology data and risk factors in basal cell carcinomas

This section describes the latest literature data on geographic distribution of lesions, distribution by age and gender groups, and depending on the location of basal cell carcinomas.

The classic and newly identified risk factors associated with basal cell carcinomas have also been described.

CHAPTER II. Pathogenesis of basal cell carcinomas

This chapter describes the biomolecular mechanisms involved in the initiation of basal cell carcinomas, as well as the genes and proteins incriminated.

CHAPTER III. Prognostic factors in basal cell carcinomas

The section included histological and molecular factors that can influence the prognosis of basal cell carcinomas.

CHAPTER IV. Clinical forms, diagnosis and treatment of basal cell carcinomas

The chapter included the latest data on clinical aspects of lesions, positive and differential diagnosis, as well as the potential of different treatment modalities of basal cell carciomas, by constantly reporting to the histopathological aspects of the lesions.

PERSONAL CONTRIBUTIONS

MOTIVATION AND THE AIM OF THE STUDY

In this study it is proposed the complex and detailed investigation of epidermal growth factor receptors EGFR, HER2 and HER3, transforming and growth factor beta (TGFβ3), one of its receptors (TGFβRIII) and Ki67 proliferation index in basal cell carcinomas , in relation to the main epidemiological and histopathological parameters of the lesions.

Immunohistochemical characterization of selected markers in relation to the aggressive criteria adopted for basal cell carcinomas can clarify aspects related to the biological behavior of lesions as well as tumor initiation aspects and local and distance progression.

CHAPTER V. MATERIAL AND METHODS

The study included a total of 778 basal cell carcinomas diagnosed over the period 2013-2017.

This study is prospective with a retrospective component, the methods used being represented by epidemiological, histopathological, immunohistochemical and statistical analysis of the obtained results.

For the immunohistochemical analysis, 53 cases were selected for quantification using the Ki67 proliferation index and composite scores that took into account the number of marked cells and the intensity of the reactions for EGFR, HER2, HER3, TGFβ3 and TGFβRIII. All the data obtained was recorded in an electronic data base, which was used for the establishment of comparison groups and for the comparative analysis of the obtained results.

CHAPTER VI. RESULTS

In the *epidemiological data analysis,* we found an increase in the incidence of basal cell carcinomas by 10.3% during the study. Most of the patients belonged to the 7th-decade of life (47.5%) and were males (54.2%), they came from the rural environment (63.6%), with head and neck lesions (84.6%) and under 2cm in size (87%).

For 55.2% of cases, associated risk factors were identified, most frequently represented by exposure to radiation (65.7%). The few cases belonged to the age younger than 50 years, for which it was found only 11 cases, representing 1.5%. Also, it was identified only one case of basal cell carcinoma under 40 years of age. The youngest patient was 37 years old, and the oldest was 88 years old with an average diagnosis age of basal cell carcinomas of 65.4 years.

The *histopathological analysis* included histological type and subtype analysis, the most common being the nodular carcinomas (71.7%), micronodular (12.2%), morpheaform (8.4%), superficial (4.2%) and metatipic (3.5%). Regarding the histopathological subtype, conventional basal cell carcinoma had an incidence of 18.2% higher compared to the incidence of all the other subtypes of nodular carcinoma.

The pTNM staging analysis indicated that most basal cell carcinomas were diagnosed in the first stage of the disease, respectively in 87% of cases, followed by stage II in 12.3% cases and stage III / IV in 0.7% cases. Among the high risk factors for basal cell carcinomas that were

identified for staging were the depth of invasion over 2 mm, Clark level ≥ IV, perineural invasion (12 cases, 1.5%).

The Clark staging analysis indicated the prevalence of stage IV lesions, for which 54% of cases were diagnosed, followed by stage V by 20.7%, stage III by 20.3%, and stage II by 5%.

In the case of invasive carcinomas at least in the subcutaneous tissue (Clark 5), particular aspects were observed in which the tumor islets distorted the architecture of the adipose tissue or the striated muscle fibers. In this study, deep invasion (Clark 5) was observed for all subtypes of basal cell carcinoma except for the superficial type, at the tumor invasion edge being usually observed stromal changes and an inflammatory or desmoplastic response.

The associated changes in the tumor stroma were represented by the myxoid transformation and the inflammatory response, the aspects being sometimes associated.

The status of the surgical resection margins indicated that in most of the analyzed basal cell carcinomas these were tumor-free, respectively in 738 cases (94.8%). In 40 cases (5.2%) we found either invasion of a lateral resection margin (20 cases) or both lateral resection (5 cases) or deep resection margin (15 cases).

The *immunohistochemical analysis* of basal cell carcinomas indicated that EGFR, HER2 and HER3 immunoexpression was identified in 90.5%, 86.7% and respectively 81.1% of analyzed carcinomas, the immunostaining being present in the tumor cell membrane. We also found significant differences in the expression of EGFR, HER2 and HER3 in relation to histological types of basal cell carcinomas analyzed.

Although the EGF receptor labeling values were superior in basal cell carcinomas in advanced stages, the aspect was statistical insignificant. In this study we found significant positive linear correlations of the EGFR, HER2 and HER3 immunoexpression.

Ki67 immunoreactions were present for all analyzed cases with the location in tumor cells nuclei. The Ki67 proliferation index (PI) revealed significant differences related to the histological type and tumor stage of basal cell carcinomas, the highest values being identified in advanced stages of adenoid and morpheaform basal cell carcinomas.

TGFβ3 and TGFβRIII immunoreactions was identified in 88.7% and 84.9% of cases in the tumor cell cytoplasm. In this study, we found significant differences in TGFβ3 and TGFβRIII immunoexpression related to the basal cell carcinoma type, and in the case of TGFβ3 in addition

with the tumor stage. High values of TGFβ3 and TGFβRIII expression were frequently associated with advanced stage adenoid and morpheaform carcinomas.

CHAPTER VII. DISCUSSIONS

Basal cell carcinoma is one of the most common malignant tumors, representing approximately 70-80% of cutaneous cancers [83]. With an annual growth incidence rate of 3-7%, the lesion present significant geographic variations in incidence, the highest values being recorded in Australia, and the smallest in Africa, the aspect being related both to the pigmentation of the skin as well as solar exposure [29].

Exposure to ultraviolet radiation, chemical and genetic factors, viral infections and immunosuppression are most commonly incriminated in the occurrence of basal cell carcinomas [7,83].

The data in the literature indicate for basal cell carcinomas a maximum incidence after 50 years with a sex ratio of M / F of 1.5-2: 1 [185]. It is indicated the predominance of basal cell carcinomas in the head and neck regions in 70-80% of cases [7].

At present, there are three major histopathological types of basal cell carcinomas with clinical correspondence, represented by nodular, superficial and infiltrative types, also being described growth patterns with variable prognosis of aggressiveness and recurrence, such as micronodular, adenoid, morpheaform , pigmented, fibroepithelial, with adnexal or squamous differentiation [83].

Welsch et al. have highlighted the correlation of the different histopathological subtypes with the depth of the invasion in a study that included a number of 100 basal cell carcinoma biopsies and found that the micronodular tumors had the highest average invasion followed by infiltrative, nodular and superficial subtypes [284].

Data in the literature indicates that a 5 mm distance from the tumor resection margin should be sufficient to avoid tumor recurrence [83].

There is evidence of EGF and receptor involvement in the development and aggressiveness of basal cell carcinomas, especially through cooperation with other mechanisms. EGF receptors studies indicate their expression in normal skin as well as variable expression in basal cell carcinomas [223].

Regarding Ki67 immunoexpression in basal cell carcinomas, the data obtained so far are controversial, from results indicating wide ranges of variation of marker expression, to the absence of correlation with histopathological parameters or association with prognostic parameters of lesions and basal cell carcinoma recurrence [140].

In the case of normal tissues and carcinomas in the initial stages, TGFβ has a suppressive effect with inhibitory effects of processes involved in local tumor development, such as inhibition of cell proliferation, induction of apoptosis, inhibition of cellular immortalization.

In the case of aggressive and invasive lesions, by activation of complex biomolecular mechanisms, such as epithelio-mesenchymal transition and angiogenesis, TGFβ activates cancer cells migration, invasion and metastasis [152].

Also, published results suggest a dual role of TGFβRIII in the sense of stimulating basal expression of TGFβ and implicitly promoting progression and metastasis and, on the other hand, implication in diminishing ligand dependent signaling by preventing ligand blockade within the TGFβRII-TGFβRI complex [171].

CHAPTER VIII. CONCLUSIONS

The *study of epidemiological data* indicated:

- the mean age at diagnosis of basal cell carcinomas was 65.4 years, 98.6% of the lesions being identified after 50 years;
- lesions predominated in males (54.2%), with a gender ratio of 1.18, and in rural areas (63.6%) with a ratio between the two environments of 1.74;
- for 55.2% of cases were identified associated risk factors, most frequently represented by exposure to radiation (65.7%), or association with family history and immunosuppression (23.9%);
- lesions were located at the head and neck level (84.6%), followed by thoracic (13.1%) and limbs (2.3%);
- lesions of less than or equal to 2cm (87%) prevailed, compared to those over 2 cm (13%).

The *histopathological analysis* of basal cell carcinomas indicated:

- nodular basal cell carcinoma was the most common histologic type (71.7%) followed by micronodular (12.2%), morpheaform (8.4%), superficial (4.2%) and metatipic (3.5%);
- Conventional basal cell carcinoma was the most common subtype of nodular carcinoma (42.4%), followed by adenoid (13.1%), cystic (9.4%), pigmented (4.9%), keratotic (1.9%);
- the pTNM staging indicated that most basal cell carcinomas were diagnosed in stage I of the disease (87%), while in Clark staging prevailed stage IV lesions (54%);
- the perineural invasion was present in 1.5% of the investigated cases, and in 5.2% of cases we found the invasion of at least a margin of resection.

The *immunohistochemical analysis* of basal cell carcinomas allowed the observations:

- it was found significant differences in the expression of EGFR, HER2 and HER3 in relation to the histological types of the analyzed basal cell carcinomas; the morpheaform type was characterized by high EGF receptor scores, the adenoid type by high EGFR and HER2 scores, and the nodular type presents the lowest values, meaning EGF receptors as proteins involved in histological aggression and histological differentiation of basal cell carcinomas;
- PI Ki67 revealed significant differences related with histological type and basal cell carcinoma tumor stage, the highest values of PI Ki67 being identified in advanced stages of adenoid and morpheaform basal cell carcinomas; there have been significant positive linear correlations of PI Ki67 with EGF receptors as well as with TGFβ3 and TGFβRIII;
- it was found significant differences in TGFβ3 and TGFβRIII immunoexpression related to the basal cell carcinoma type, and in the case of TGFβ3 also in relation to the tumor stage;
- the high values of TGFβ3 and TGFβRIII expression were frequently associated with advanced stage of adenoid and morpheaform carcinomas;
- we observed significant positive linear correlations of TGFβ3 and TGFβRIII as well as TGFβ3 related to EGFR and HER2;
- the results obtained in this study indicate the involvement of the markers analyzed in the aggressiveness of the basal cell carcinomas, which supports the inclusion in the target group with therapeutic potential for these lesions.

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