UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA

DOCTORAL SCHOOL

PhD THESIS

Study of aggressive factors in basal cell carcinoma- Clinical, histopathological and immunohistochemical correlations

ABSTRACT

PhD COORDINATOR:

PROF. UNIV.DR. SIMIONESCU CRISTIANA

PhD STUDENT:

ENACHE ANDREEA-OANA

CRAIOVA 2019

SUMMARY

INTRODUCTION

Skin cancers represent about 90-95% of all cancers with this location. Given the increase in solar activity in recent years, the continuous reduction of the ozone layer and the excessive sun exposure of young people, it is estimated that there will be significant increases of skin cancers globally [155, 227].

Basal cell carcinoma (BCC) accounts for approximately 80% of non-melanoma skin cancers, and is the most common malignancy in white people. It is a slow growing, locally invasive tumor, with high healing rates and excellent prognosis when it is diagnosed in early stages [136, 155].

Despite this relatively benign behavior, there are aggressive clinical forms of BCC that can cause extensive local tissue damage, with a significant impact on patients' quality of life. The challenge is to establish a correct diagnosis in a less invasive manner [44, 248].

Dermatoscopy offers a detailed picture of the surface structures, increases the sensitivity and specificity in the diagnosis of basal cell carcinoma, being an effective method of screening.

However, the confirmation of the diagnosis required the histopathological examination of a cutaneous biopsy [102, 228]. The histological diagnosis and classification of basal cell carcinomas are essential for determining the tumor type and its biological behavior.

This study aims the complex epidemiological, histopathological and immunohistochemical investigation of the basal cell carcinomas in order to establish statistical relations, supporting the identification of the high risk types of basal cell carcinoma.

Keywords: basal cell carcinoma, dermatoscopy, histopathological type, COX-2, EGFR, HER 2, Ki67, p53, Bcl-2, MMP-2 and TIMP-2

CHAPTER I. Epidemiology 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 anatomical distribution of basal cell carcinomas. The risk factors predisposing to the development of basal cell carcinomas were also described.

CHAPTER II. Cutaneous carcinogenesis. This chapter describes the mechanisms involved in cutaneous carcinogenesis of basal cell carcinomas, as well as the genes and proteins incriminated.

CHAPTER III. Diagnostic approach and prognostic factors in basal cell carcinomas

The section included the most recent data regarding the clinical aspects of the lesions, the positive and differential diagnosis, as well as the clinical and histological factors that may influence the tumor prognosis.

PERSONAL CONTRIBUTIONS

MOTIVATION AND THE AIM OF THE STUDY

In this study it is proposed the complex and detailed investigation of cutaneous carcinogenesis, focusing on basal cell carcinomas, as well as identifying the factors involved in the unfavorable evolution of some of these tumors, in order to identify the possible prognostic and therapeutic targets.

CHAPTER IV. MATERIAL AND METHODS

The study included a total of 390 basal cell carcinomas diagnosed over the period 2015- 2016.

The clinico-epidemiological study took into account the following parameters: sex, age and profession of the patients; the environmental origin; the onset mode; significant personal pathological background; the clinical diagnosis; the tumor location; time elapsed since the onset of the tumor; particular aspects related to evolution; the dermatoscopic pattern.

The histopathological study of the investigated basal cell carcinomas, aimed to identify the main histopathological parameters related to the prognosis: the tumor growth pattern, the depth of the invasion, the perineural invasion, the associated changes, the status of the resection limits.

For the *immunohistochemical* study, a number of 50 cases were selected, with the diagnosis of nodular or infiltrating basal cell carcinoma, for which we aimed to evaluate the expression of some markers involved in skin carcinogenesis, such as COX-2, EGFR, HER 2, Ki67, p53, was sought. Bcl-2, MMP-2, TIMP-2.

The results obtained were statistically analyzed and statistical tests were used to assess the differences between the immunoreactivity scores obtained for each tumor, considering a significant difference at a level of $p < 0.05$.

CHAPTER V. RESULTS

In the *clinico-epidemiological* data analysis, we found that the majority of patients belonged to the 7th decade of life (39.2%) and were females (57.7%), they came from the rural environment (55.9%), with lesions predominantly at the cephalic extremity level (82.8%).

For 60.76% of the cases, clinical manifestations of skin aging were identified (accentuated wrinkles, cutis rhomboidalis nuchae, solar lentigo etc). All patients had phototype II and III, underlining the importance of individual susceptibility to cancer. In our study we found all

clinical forms of basal cell carcinoma described in the literature. The pearly BCC was the most commonly encountered (51.6%), followed by the nodular form (19.5%) and cicatricial basal cell carcinoma. *Dermatoscopically,* 15.1% of the cases were non-pigmented basal cell carcinomas, the rest being pigmented (40%), slightly pigmented (36.9%) and strongly pigmented (8%). Regarding the presence of the elements characteristic for the diagnosis of basal cell carcinoma from the dermatoscopic point of view, the arborizing vessels were the most frequently encountered (180 cases).

The *histopathological* analysis indicated that the most common histologic type was nodular basal cell carcinoma (62.3%), followed by micronodular (11%), superficial (10.8%) and basosquamous carcinoma (0.8%). Clark's staging analysis indicated the prevalence of stage IV lesions (41.3%), followed by stage III (39%), stage V with 14.1% and stage II with 5.6% of cases. The perineural invasion was present in 3 cases. The aspect was identified in tumors with large dimensions, over 7 cm in maximum diameter, associated with deep invasion, in the bone or striated muscle tissue. In 1 case we observed aspects of vascular invasion. The status of the surgical resection margins indicated that in most of the analyzed basal cell carcinomas these were tumor-free, respectively 347 cases and showed tumor invasion in 43 cases. In our study, the tumor invasion was identified at the level of a single surgical resection margin for a number of 31 cases (7.9%), of which in 14 cases the deep border was invaded and in 17 cases one of the lateral limits. The tumor invasion was identified at the level of two resection limits in 11 cases (2.8%), and for 1 case (0.2%) the invasion of all three surgical safety margins was present. We noticed a number of changes associated with basal cell carcinoma such as: ulceration (23.07%), senile elastosis (64.10%), actinic keratosis (6.41%) and tumor calcifications (0.51%).

Immunohistochemical analysis of basal cell carcinomas indicated that bcl-2, EGFR, HER2, TIMP2 immunoexpression was identified in 76%, 74%, 48%, and 52% respectively of the analyzed tumors, with progressively higher SCM values with increasing depth of tumor invasion, for both investigated forms, the differences in scores being statistically significant with both Clark level and tumor type. We also observed statistically significant positive linear correlations of p53, COX2 and Bcl-2 ($p < 0.001$, Pearson test), as well as Ki67, EGFR and HER2. Although the values of COX2 receptor markers were higher in the basal cell carcinomas in advanced stages, the appearance was statistically insignificant. Immunostaining for MMP-2 was identified in 44% of the investigated cases, with a low reaction intensity for all invasion grades in both investigated forms but with insignificant differences in MMP2 scores in relation to tumor type and stage.

CHAPTER VI. DISCUSSIONS

Basal cell carcinoma accounts approximately 80% of all skin cancers. There have been reported geographical variations in the incidence of basal cell carcinoma: the highest values being reported in Australia, where there are over 1200 cases⁄100 000 inhabitants / year [224].

The incidence of basal cell carcinoma continues to increase globally. It is estimated that approximately 2.5 million of new cases are diagnosed each year in the US, with an annual growth rate of about 2%. In Europe the annual growth rate has been 5% in the last decades [155, 227].

It is known that actinic radiation is the main etiological factor, with over 80% of the basal cell carcinomas having localization at the cephalic extremity level, like the data obtained in our study [75].

The histological diagnosis and classification of basal cell carcinomas are essential to determine the type of tumor and its biological behavior.

Currently, three major histopathological types of basal cell carcinomas are accepted, with clinical correspondence, represented by nodular, superficial and infiltrative types, also being described multiple growth patents, with different rates of aggression and recurrence, such as micronodular, adenoid, morphiform, pigmented, fibroepithelial, with adnexial or squamous differentiation.

Perineural invasion is associated with large, aggressive tumors, and the risk of tumor recurrence at 5 years is higher [144]. This underlines the importance of tumor excision with the control of surgical resection limits and the long-term patient monitoring [144].

There are evidences that COX-2 may be involved in the pathogenesis of non-melanoma skin cancers[70]. Although the role of COX-2 in skin carcinogenesis is unclear, it is believed that COX-2 expression may be a result of p53 mutation [264, 119]. Therefore, COX-2 modulation is a promising area investigated by several research groups.

Oncoproteins and suppressor genes, which are responsible for protein expression: p53, bcl-2 and the proliferation marker Ki-67, play a significant role in the development and progression of non-melanoma skin cancers [134, 296]

Studies carried out on epidermal growth factor receptor (EGFR), HER2, HER3 and HER4 indicating their expression in the case of normal skin, their role in the development of basal cell carcinomas being not fully understood [240].

MMP-2 is largely secreted by keratinocytes and tumor cells of basal cell carcinomas [204]. Interaction between stromal fibroblasts and tumor cells of basal cell carcinomas affects MMP-2 expression derived from fibroblasts, suggesting a significant effect of this interaction on cancer development.

TIMP-2 contributes significantly to the biological behavior of skin tumors by their significantly ability to inhibit metastasis [288].

CHAPTER VII. CONCLUSIONS

- The clinico-epidemiological study indicated that the average age of diagnosis of the patients was 70.3 years, with a maximum incidence between 60 and 80 years (61.79%), tumors being found predominantly at females (57.7 %);
- The lesions were predominantly located at the cephalic extremity level (84.6%), followed by thoracic (15.1%) and limbs (2.3%);
- The most commonly encountered clinical form was the pearly BCC (51.6%), followed by the nodular form (19.5%) and the scar plan type (9%);

- Dermatoscopy showed: the presence of ulceration (15.3%); arborizing vessels (46.1%); multiple blue/gray globules of different sizes (24.3%); the presence of maple leaf-like areas on the periphery (30.7%); spoke-wheel-like structures (17.4%); brown and gray granular pigmentation (13.8%);
- The histopathological study indicated that nodular basal cell carcinoma was the most common type (62.3 %) followed by micronodular (11%), superficial (10.8%) and basosquamous carcinoma (0.8%);
- Perineural invasion was identified in 0.8% of the investigated cases, associated with the infiltrative and sclerosing type and the vascular invasion was identified in 0.3% of the investigated cases, associated with the infiltrative type.
- Clark's staging analysis indicated the predominance of stage IV lesions (41.3%), followed by stage III (39%), stage V with 14.1% and stage II with 5.6% of cases;
- For 7.9% of the cases we found the invasion of a lateral resection limit, of both lateral resection limits in 2.8% of the cases, and in 0.2% of the cases the invasion of all three surgical safety margins was present.
- The immunohistochemical study followed the expression of the cell cycle markers (COX2), apoptosis (p53, bcl-2), proliferation (ki67), cell growth (EGFR, HER2) and stromal markers (MMP-2, TIMP-2).
- The analysis of COX2 expression was relevant in 88% of the analyzed cases, with progressively higher SCM values with increasing depth of tumor invasion, for both investigated forms, but the scores differences were statistically insignificant, with both Clark level and tumor type.
- The p53 analysis revealed positivity in 74% of the analyzed cases, with high SCM values regardless of the depth of invasion; statistical analysis indicated significant differences in p53 scores relative to Clark level and at the limit of statistical significance with tumor type, high positive scores being associated with infiltrative tumors with a high Clark level.
- The analysis of bcl-2, EGFR, HER2, TIMP2 indicated positivity in 76%, 74% 48% and 52% of the investigated cases, with progressively higher SCM values with increasing depth of tumor invasion for both investigated forms, the differences in scores being statistically significant with both Clark level and tumor type.
- Immunostaining for ki67 was identified in 86% of the investigated cases, with progressively higher IP values with increasing depth of tumor invasion for both investigated forms, the distribution of average values IPKi67 indicating significantly higher differences in

infiltrative carcinomas ($p < 0.001$, Anova test) being in advanced stages ($p < 0.001$, Anova test)

- Immunostaining for MMP-2 was identified in 44% of the investigated cases, with a low reaction intensity for all invasion grades in both investigated forms, with no significant difference of MMP-2 in relation to the type ($p = 0.121$, χ^2 test) or tumor stage ($p = 0.096$, test χ^2).

SELECTIVE REFERENCES

44. Chinem VP, Miot HA. Epidemiology of basal cell carcinoma. An Bras Dermatol. 2011;86(2):292–305.

75. Farhi D, Dupin N, Palangié A, et al. Incomplete excision of basal cell carcinoma: Rate and associated factors among 362 consecutive cases, Dermatologic Surgery, 2007; 33(10):1207–1214.

102. Haws AL, Rojano R, Tahan SR, et al. Accuracy of biopsy sampling for subtyping basal cell carcinoma, Journal of the American Academy of Dermatology, 2012;66(1):106–111.

119. Karagece Yalçin U, Seçkın S. The expression of p53 and COX-2 in basal cell carcinoma, squamous cell carcinoma and actinic keratosis cases. Turk Patoloji Derg. 2012;28(2):119-27.

134. Kramer E, Herman O, Frand J, et al. Ki67 as a biologic marker of basal cell carcinoma: a retrospective study. Isr Med Assoc J. 2014;16(4):229-232.

136. Kumar S, Mahajan BB, Kaur S, et al. A study of Basal cell carcinoma in South asians for risk factor and clinicopathological characterization: a hospital based study. J Skin Cancer. 2014;2014:173582.

144. Leibovitch I, Huilgol S, Selva D, et al. Basal cell carcinoma treated with Mohs surgery in Australia III. Perineural invasion. J Am Acad Dermatol. 2005;53:458–463.

155. Lomas A, Leonardi-Bee J, Bath-Hextall F. A systematic review of worldwide incidence of nonmelanoma skin cancer. Br J Dermatol 2012; 166:1069–1080.

157. Machan M, Kroh JM, Hunt E, et al. Letter: basal cell carcinoma with vascular invasion. Dermato

204. Pittayapruek P, Meephansan J, Prapapan O, et al. Role of Matrix Metalloproteinases in Photoaging and Photocarcinogenesis. Int J Mol Sci. 2016; 17(6): 868.l Online J. 2012;18(4):13.

224. Richmond-Sinclair NM, Pandeya N, Ware RS et al. Incidence of basal cell carcinoma multiplicity and detailed anatomic distribution: longitudinal study of an Australian population. J Invest Dermatol 2009; 129:323–328

227. Rogers HW, Weinstock MA, Feldman SR et al. Incidence estimate of nonmelanoma skin cancer (keratinocyte carcinomas) in the U.S. population, 2012. JAMA Dermatol 2015; 151:1081–1086.

264. Subbaramaiah K, Altorki N, Chung WJ, et al. Inhibition cyclooxygenase-2 gene expression by p53. J Biol Chem 1999, 274:10911-10915.

228. Roozeboom MH, Mosterd K, Winnepenninckx VJL, et al. Agreement between histological subtype on punch biopsy and surgical excision in primary basal cell carcinoma, JEADV, 2013;27(7):894–898.

240. Schneider MR, Yarden Y. The EGFR-HER2 module: a stem cell approach to understanding a prime target and driver of solid tumors. Oncogene. 2016; 35: 2949–2960.

248. Shrivastava R, Singh KK, Shrivastava M. Soft tissue metastasis in basal cell carcinoma. Indian J Dermatol. 2007;52:206–208.

288. Wagner SN, Ockenfels HM, Wagner C, et al. Differential expression of tissue inhibitor of metalloproteinases-2 by cutaneous squamous and basal cell carcinomas. J Invest Dermatol. 1996;106(2):321-326.

296. Wikonkal NM, Berg RJ, van Haselen CW, et al. bcl-2 vs p53 protein expression and apoptotic rate in human nonmelanoma skin cancers. Arch Dermatol. 1997; 133(5):599-602