UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA
DOCTORAL SCHOOL

PHD THESIS
Abstract

PROGNOSTIC FACTORS IN PRECURSORS LESIONS AND CUTANEOUS SQUAMOUS CELL CARCINOMA. CLINICO-EPIDEMIOLOGICAL, HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY

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CRAIOVA 2017

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INTRODUCTION

The current prevalence of cutaneous cancer is alarming [223], in the United States over one third of all cancers are nonmelanoma skin cancers [379]. Early detected and treated, squamous carcinomas have a 95% healing rate [52]. However, neglected cases can cause local tissular destruction and can metastasize [10]. Moreover, 3-year after the initial diagnosis, the cumulative risk of developing a second lesion is approximately 18% [419].

Many of the deaths caused by the disease can be prevented by treating the actinic keratosis- the earliest manifestations of squamous cell carcinoma. An actinic keratosis may regress, persist, or progress to squamous cell carcinoma, while being impossible to assess the individual evolution of each lesion. According to the American Academy of Dermatology, [113] it is estimated that 60% of people over the age of 40 have at least one actinic keratosis. Although it is estimated that only 0.1-10% of the actinic keratoses can progress to squamous cell carcinoma, their presence serves as an indicator for identifying the degree of risk of a population to develop a solar radion induced condition like squamous cell carcinoma [84].

Actinic keratosis proves to be laborious because it is difficult to clinically differentiate actinic keratosis from squamous cell carcinoma. It is therefore essential for clinicians to obtain histopathological information when they are unable to clearly identify the nature of the lesion.

The present study aims a complex assessment of precursor lesions and squamous cell carcinoma using clinical-morphological and immunohistochemical techniques, in order to select specific markers that can be used in identifying the lesions with risk of progression and metastasizing .

Key words: cutaneous squamous cell carcinoma, precursor lesions, actinic keratosis, Bowen's disease, immunohistochemistry, prognostic factors.

CHAPTER I.EPIDEMIOLOGY OF PRECURSOR LESIONS AND CUTANEOUS SQUAMOUS CELL CARCINOMAS - This chapter selects from the literature data concerning global incidence of precursor lesions and cutaneous squamous cell carcinoma.
CHAPTER II - RISK FACTORS FOR PRECURSOR LESIONS AND CUTANEOUS SQUAMOUS CELL CARCINOMAS presents data about the risk factors involved in cutaneous carcinogenesis: skin phototype, ultraviolet radiation exposure, immunodeficiency, genetic factors, etc.

CHAPTER III. PATHOGENESIS OF THE PRECURSOR LESIONS AND CUTANEOUS SQUAMOUS CELL CARCINOMAS - presents the mechanisms through which UV radiation exposure determines a series of intricate genetic events that subsequently determine the development of actinic keratosis and cutaneous squamous cell carcinoma.
CHAPTER IV. PROGNOSIS FOR THE PRECURSOR LESIONS AND CUTANEOUS SQUAMOUS CELL CARCINOMAS – this chapter examines the evolution of actinic keratosis in the sense of spontaneous regression, persistence or progression to squamous cell carcinoma [28, 388,163, 299]. Prognosis is influenced by the moment of diagnosis, immune status, size of the lesion, depth of invasion, Clark Level, perineural invasion, tumor grade (poorly differentiated/ undifferentiated), and anatomical localization [54].

PERSONAL RESEARCHES

PURPOSE AND SPECIFIC OBJECTIVES The present study aims at an evaluation of cutaneous carcinogenesis and identification of factors involved in the unfavorable progression of some of the analyzed lesions, trying to select the most reliable markers that characterize skin carcinogenesis, the goal been to identify possible molecular targets in order to apply early and differentiated therapies.

CHAPTER V. MATERIAL AND METHODS. Material - was represented by the clinical observation records, the anatomo-pathological registers, the surgical excision samples, the paraffin blocks and the histological slides from the studied cases. The analysis of cutaneous lesions was done statistically, clinically-epidemiologically, histopathologically by usual techniques with standard staining with Hemalaum-Eosin and immunohistochemical, the antibodies used were: P53, Bcl-2, Ki67, p16, p21, Cyclin D1, CD44, E-cadherin and N-cadherin.

CHAPTER VI. **and VII.** RESULTS AND DISCUSSION- in these chapters the obtained data were analyzed and compared with those from the literature.
Clinical-epidemiological study revealed for both lesional categories the existence of a similar age and gender incidence, with patients in the 70-79 interval being more frequent affected, with males being more frequently affected than women. Topography of lesions - revealed a maximum frequency in head area, more frequent in the cheek area - for both studied lesions. Actinic keratosis cases had

varied morphological aspects, the most common being the classical type - encountered in 43.7%, while the less common being the Bowenoid type, similar to the literature (3, 8). The investigated squamous cell carcinomas also featured a wide variety of clinical aspects: papules, plaques, smooth or hyperkeratotic nodules, etc. Metastases were identified in 12 cases. Tumors were generally asymptomatic. The lesions were in all cases unique, with sizes ranging from 1 mm to 52 mm.

HISTOPATHOLOGICAL STUDY - Actinic keratosis was the major part (84.4%) of squamous cell carcinoma precursor lesions, and 22.6% of the analyzed cases. The lesional growth patterns of actinic keratosis were most common the conventional type in 41 cases (30.6%), the less common being pigmented type in 5 cases (3.7%). From the histological point of view, the most numerous were KIN I lesions- diagnosed in 82 cases, followed by KIN II lesions in 38 cases and KIN III lesions in 14 cases. The histopathological study of the 435 squamous carcinomas revealed the typical nonkeratinized form in 2/3 of all the squamous carcinomas analyzed, the carcinomas being moderately differentiated in more than half of the cases, well-differentiated in about a quarter of the cases, and poorly differentiated in the remaining cases. The vascular-lymphatic invasion was identified in 3.2% of the casuistic, the perineural invasion in 22.5%, the surgical resection limits had tumor invasion in only 43 cases. The analysis of regional lymph node involvement (pN category) indicated the presence of metastatic adenopaty in 2.8% of cases and its absence in 88.5% of cases. The evaluation of the pTNM staging included 38 cases corresponding to stage 0, 205 cases corresponding to stage I, 177 cases corresponding to stage II and 15 cases corresponding to the 3rd stage of the disease. Depending on the Breslow index, most cases were stage III and II (35% and 33.4% respectively), while 4.4% corresponded to Breslow I stage.
Depending on the Clark index - most of the carcinomas were invasive in the reticular dermis, respectively 207 cases (47.6%).

IMMUNOHISTOCHIMIC STUDY - For the selected 91 cases, we have observed the expression of markers involved in: apoptosis (p53 and Bcl-2), proliferation (Ki67), cell cycle (p16, p21 and D1 cyclin), and intercellular adhesion (CD44, E-cadherin and N-cadherin).

Analysis of p53 expression revealed a positive reaction in 74 cases (83.5%), of which 19 cases of actinic keratosis and 53 cases of squamous cell carcinoma.

The statistical analysis showed significant higher differences in p53 expression in squamous cell carcinomas compared to KIN lesions ($p = 0.000$, square chi test), as well as in moderately or poorly differentiated forms of squamous cell carcinomas compared to well differentiated carcinoma ($p = 0.000$, test chi square). We did not find differences in p53 expression relative to the tumor stage ($p = 0.059$, qui square test).

Bcl-2 expression was present only in the 22 cases of actinic keratosis. We did not find statistical associations of bcl-2 and p53 expression ($p = 0.322$, Pearson test).

The Ki67 expression analysis revealed the positive reaction in 76 cases (83.5% of our casuistry), respectively in 20 cases of actinic keratosis, in the two cases of Bowen's disease and in 54 cases of squamous cell carcinoma. The Pearson test indicated a positive linear correlation of the distribution of the percentage values of the Ki67 and p53 markers, which was statistically significant (p = 0.000, Pearson test). We did not find statistical relationships between the Ki67 proliferation index and the bcl-2 antiapoptotic index (p = 0.929, Pearson test).

Analysis of p16 expression revealed the positivity of the reaction in 61 cases (67%), of which 16 cases of actinic keratosis, 2 cases of Bowen's disease and 43 cases of squamous cell carcinoma. The statistical analysis showed significantly higher differences in p16 expression in carcinoma compared to KIN lesions (p = 0.000, square chi square) as well as in stage II/III carcinoma compared to those from stage I (p = 0.029, test chi square). We did not find differences in p16 expression relative to tumor differentiation (p = 0.091, qui square test).

Analysis of p21 expression revealed the positivity of the reaction in 65 cases (71.4%), of which 23 cases of actinic keratosis, 2 cases of Bowen's disease and 40 cases of squamous cell carcinoma. The statistical analysis indicated significant differences in p21 expression relative to the type and degree of carcinoma differentiation (p = 0.000, qui square test). Thus, the high scores of p21 have been identified only for Bowen disease and well-differentiated carcinoma. Likewise, although high p21 scores were only observed in early stages carcinoma, we did not find a statistical correlation of p21 relative to the tumor stage (p = 0.536, quadratic square test).

Analysis of Cyclin D1 expression revealed the positive reaction in 65 cases (71.4%), of which 19 cases of actinic keratosis and 46 cases of squamous cell carcinoma with varying degrees of differentiation. Statistical analysis indicated that high scores of cyclin D1 were only identified for carcinoma, while being absent for KIN lesions (p = 0.014, square test). We did not find differences in Cyclin D1 expression relative to tumor stage P = 0.682, quadratic square test).

Analysis of CD44 expression revealed the immunoreaction positivity in 84 cases (92.3%), of which 28 cases of actinic keratosis and 56 cases of squamous cell carcinoma. The statistical analysis showed significantly higher differences in KIN lesions compared to carcinoma, like in well differentiated carcinoma compared to moderate and poorly differentiated form (p = 0.005, square test). We did not find any statistical associations between the CD44 expression and the tumor stage (p= 0,601, quadratic square test). Analysis of E-cadherin expression revealed a positive reaction in 72 cases (79.1%), of which 28 cases of actinic keratosis, 2 cases of Bowen's disease and 42 cases of squamous cell carcinoma. The statistical analysis showed significant higher differences in KIN lesions compared to carcinoma and in well differentiated carcinoma compared to moderately differentiated (p = 0.000, qui square test). We have not found statistical associations of E-cadherin expression with tumor stage.

Analysis of N-cadherin expression revealed the positivity of the reaction in 26 cases (28.5%), of which 13 cases of actinic keratosis and 13 cases of squamous cell carcinoma.

CHAPTER VIII. CONCLUSIONS

Among the most important conclusions: Clinical-epidemiologic - both lesional categories affected similar age groups, with a maximum incidence in 6th and 7th decade, male being the most affected, the head area being the most frequently involved.

- The clinical examination of pre-invasive lesions revealed the predominance of the classical type (43.7%), the less common being pigmented and bowenoid type lesions; Squamous cell carcinomas were more frequent ulcerated (36.6%). The dimensions of the actinic keratosis lesions were between a few millimeters and 1.8 cm; those of squamous carcinomas were less than 2 cm in 56.3% of cases, and over 2 cm in 43.7%.
- The histopathological analysis revealed in the case of actinic keratosis that the most common type was the conventional one (30.6%), regarding the histological grade, the most frequent were KIN I (61.1%), and the rarest KIN III (10.5%).

For the 435 squamous cell carcinoma of the skin we mention the following:

- non-keratinized conventional squamous cell carcinomas were the most frequent histopathological form (66.7%), desmoplastic carcinoma being the least common - 5 cases (1.1%). Moderately differentiated forms of non-keratinized squamous cell carcinoma were the most common (58.6%), vascular-lymphatic invasion was present in only 14 cases (3.2%) and perineural invasion was identified in 98 cases (22.5%); Metastatic adenopaty was encountered in only 2.8% of cases. Evaluation of pTNM staging was performed for 397 cases of invasive carcinoma and 38 cases of in situ carcinoma, of which 38 cases corresponded to stage 0, 205 cases corresponded to stage I, 177 cases corresponded to stage II and 15 cases corresponded to the third stage of the disease.
- from the Breslow index point of view, most tumors (35%) corresponded to Breslow III, followed in the order of Breslow II tumors (33.4%), and the most rare were Breslow II and I (10% and 4.4% respectively).
- regarding Clark index - most carcinoma were invasive in the reticular dermis (47.6%), followed in the order of frequency by carcinomas with invasion of the papillary dermis (23.5%), the most rare were the carcinomas with incipient invasion in the papillary dermis (11.7%).
- the study indicated significant association of the Breslow V index with tumor stages II and III, as well as the association of tumor stage I with the Breslow I-IV index ($p = 0.000$, and square)

Immunohistochemical analysis of precursor lesions and cutaneous squamous cell carcinomas revealed:

- Over-expression of p53 was significantly superior in carcinomas compared to KIN lesions, as well as in moderately or poorly differentiated forms of squamous carcinomas compared to well-

differentiated;

- Positive Bcl-2 responses were identified in only a limited number of KIN lesions, being negative for carcinoma, which argue for a secondary role of anti-apoptotic mechanisms in the progression of these lesions;
- The Ki67 average proliferation index was significantly superior in carcinoma compared to KIN lesions, as well as in moderate and poorly differentiated carcinoma from the advanced stages ($p = 0.000$, Anova test);
- The high prevalence of p53 oncoprotein (86.8%) and Ki67 antigen (88.4%) in cutaneous squamous carcinoma support their pathogenic role in cutaneous carcinogenesis;
- P16 immune responses were significantly higher in carcinoma compared to KIN ($p = 0.000$, chi square test), as well as in advanced stage carcinoma compared to those in the initial stages ($p = 0.029$, chi square test);
- p16 expression was independent of p53 one;

- High scores of p21 immunoreactions were only identified for Bowen disease and well-differentiated carcinomas;
- Cyclin D1 immunoexpression was significantly superior in carcinomas compared to KIN lesions;
- N-cadherin immunoexpression was significantly superior in carcinoma compared to KIN lesions;
- Cadherin expression alteration is associated with loss of cell differentiation, the acquisition of an invasive phenotype and an unfavorable prognosis; The loss of E-cadherin expression and the gain of N-cadherin expression are events that remind of switching of cadherins in the epithelio-mesenchymal transition process.

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