

The involvement of EGFR, HER2 and HER3 in the basal cell carcinomas aggressiveness

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Abstract

Basal cell carcinomas (BCCs) are the most common malignant skin tumors, with variable prognosis and recurrence rates, depending on histopathological subtypes. The study analyzed the immunoexpression of epidermal growth factor receptors (EGFRs) in 53 cases of nodular, adenoid and morpheaform BCCs in relation to clinico-pathological associated parameters. We found significant differences in the expression of EGFR, HER2 and HER3 reported to histological BCC types. The nodular type presented the weakest expression of EGFRs, while the morpheaform type had a high expression of all receptors and the adenoid type an increased expression only in case of EGFR and HER2. This study supports the involvement of EGFR, HER2 and HER3 in BCC aggressiveness of and in tumor differentiating towards different histological subtypes.

Keywords: basal cell carcinoma, aggressiveness, EGFR, HER2, HER3.

Introduction

Basal cell carcinoma (BCC) is the most common human malignancy, representing about 70–80% of cutaneous malignant tumors and appears to have the origin in interfollicular basal cells or keratinous cells from hair follicles or sebaceous glands [1–4]. Although has a local aggressive behavior, the metastatic BCC rate is low [1–3]. The exposure to ultraviolet radiation, chemical and genetic factors, viral infections and immunosuppression are most commonly implicated in the occurrence of BCC [1].

There were numerous classifications of BCC over time, which have tried to take into account the clinical and histopathological aspects of tumors. Currently, there are three major histological types of BCC with clinical correspondence, represented by the nodular, superficial and infiltrative types [2, 5]. Also, are described numerous growth patterns with variable prognosis and aggression and recurrence rates, as are the micronodular, adenoid, morpheaform, pigmented, fibroepithelial types or with adnexal or squamous differentiation types [2, 5].

The particular biological behavior of BCC compared to other malignant tumors and the absence of precursor lesions, supported the research of biomolecular mechanisms underlying the initiation and histological differentiation of BCC, for the efficient and differentiated therapy [6–8]. In this context, Ras–mitogen-activated protein kinase (MAPK) and Hedgehog pathways, peroxisome proliferator-activated receptor-gamma (PPAR- γ) and transforming growth factor-beta (TGF- β) signaling, p53 alterations and intercellular interactions appear to be involved [7, 8]. Together with these biomolecular alterations, there is

evidence of epidermal growth factor receptors (EGFRs) involvement in the development and aggressiveness of BCC, especially considering the cooperation with other mechanisms [9–11].

In this study, we analyzed the immunoexpression of EGFR, HER2 and HER3 in relation to clinicopathological parameters of BCC.

Materials and Methods

In this study, we analyzed 53 basal cell carcinomas from patients admitted and operated in the Clinics of Dermatology and Plastic Surgery, Emergency County Hospital of Craiova, Romania, during 2013–2015. The lesions were diagnosed and histopathologically classified in the Laboratory of Pathology of the same Hospital, based on criteria established by the working group for non-melanocytic tumors of the skin within *American Joint Committee on Cancer* (AJCC) [12]. Therefore, the surgical specimens were fixed in 10% neutral buffered formalin and then processed by the classic histopathological technique consisting on paraffin embedding and Hematoxylin–Eosin (HE) staining.

The clinicopathological analysis characterized aspects related to patients' gender and age, and also to tumor location, size, histological type and tumor stage. For the immunohistochemical (IHC) analysis, serial sections were used, which were processed by Biotin-Free Catalyzed Amplification System CSA II (Dako, Redox, Romania, code K197) in the case of EGFR and HER3, and Labeled Streptavidin–Biotin (LSAB)+ System–Horseradish peroxidase (HRP) (Dako, Redox, Romania, code K0675) for

HER2/neu, with the using of 3,3'-Diaminobenzidine tetrahydrochloride as chromogen (Dako, Redox, Romania, code K3468) for the detection of the signals. The sections were incubated with primary antibodies that are found in Table 1 and for the validation of the reactions were used positive and negative external controls.

Table 1 – Antibodies and protocols used in the study

Antibody	Clone	Dilution	Pretreatment	External positive control
EGFR	H11/Dako	1:300	–	Placenta
EGFR2 (HER2/neu)	Polyclonal/Dako	1:75	Microwaving in citrate buffer, pH 6	Breast cancer
EGFR3 (HER3)	DAK-H3-IC/Dako	1:100	Microwaving in Tris-EDTA buffer, pH 9	Small intestine

EGFR: Epidermal growth factor receptor; HER2: Human EGFR2; HER3: Human EGFR3; EDTA: Ethylenediaminetetraacetic acid.

The quantification of IHC reactions was performed using the positivity composite scores resulting by the multiplying of the reactions intensity scores (1: weak, 2: moderate, 3: intense) with the scores of labeled cells percentage (1: less than 40%, 2: 40–60%, 3: over 60%). For the statistical analysis, the scores were considered low for values between 1–4 and high for 6–9 values.

Image acquisition was performed using Nikon Eclipse E600 microscope and Lucia 5 software. Since the immunostainings were observed in the non-epithelial elements, in order to increase the quantification accuracy, the analyzed images resulted from the processing of the acquired images in sense of removal of stromal areas through image analysis software (Image ProPlus 7 AMS, Media Cybernetics, Inc., Buckinghamshire, UK).

For the statistical analysis, there were used χ^2 (chi-square) and Pearson's tests, using Statistical Package for the Social Sciences (SPSS) 10 software, the results being considered significant for p -values <0.05.

The local Ethical Committee approved this study, and written informed consent was obtained from all the patients.

Results

In this study, the basal cell carcinomas predominated in patients over 50 years (79.2%), in males (67.9%), localized in the head and neck regions (83%) and size less than 2 cm (56.6%). Most of the tumors were represented by the nodular type (52.8%), in the stage I of disease (56.6%) (Table 2).

Table 2 – Clinical and histological aspects of the investigated group

Clinico-pathological parameters	Values for the investigated group
Age [years]	≤50: 11, >50: 42
Gender	Males: 36, females: 17
Tumor location	Head & neck: 44, trunk & members: 9
Tumor size [cm]	≤2: 30, >2: 23
Histological type	Nodular: 28, adenoid: 16, morpheaform: 9
Stage	I: 30, II: 19, III: 3, IV: 1

EGFR, HER2 and HER3 immunoreactions were found in the cytoplasm and membrane of tumor cells in 90.5%, 86.7% and 81.1% of analyzed BCC cases. The immunostainings were observed also in some stromal elements represented by fibroblasts, lymphocytes, plasma cells or endothelial cells. Also, in the tumoral adjacent normal skin, we found diffuse reactions, with variable intensity of the analyzed markers in the sebaceous glands, sweat glands and hair follicles. In the epithelium thickness, especially in the case of EGFR and HER2, we found in the inferior half, faint reactions as intensity and number of labeled cells.

For BCC, the EGFR immunoreactions indicated variable intensity, the number of labeled cells being between 35–85%, with a mean value of 58.9±11.6 and an average composite score of 5.6. EGFR negative cases belonged to the nodular type. The morpheaform and adenoid types revealed moderate or increased intensity, while the number of labeled cells was between 65–85% (mean value 69.4±9.5) and 40–75%, respectively (mean value 69.4±9.5), with composite average scores of 7.7 and 6.2. By comparison, in cases of nodular BCC, the intensity of the reactions was predominantly low or moderate, the number of labeled cells being between 35–65% (mean value 53.3±9.5) and with a mean score of 4.3 (Table 3; Figure 1, A–C).

Table 3 – EGFR, HER2 and HER3 immunoexpression significance

Histological type	Labeled cells [%] / Composite score (average values)		
	EGFR	HER2	HER3
Nodular	53.3±9.5 / 4.3	57.7±10.9 / 3.2	48.6±11.6 / 2.4
Adenoid	69.4±9.5 / 6.2	63±10.7 / 6.5	50.8±10.8 / 2.8
Morpheaform	69.4±9.5 / 7.7	73.5±8.9 / 8.3	71.8±9.9 / 7.2
p -value (chi-square test)	<0.01	<0.001	<0.001

EGFR: Epidermal growth factor receptor; HER2: Human EGFR2; HER3: Human EGFR3.

In case of HER2, BCC immunostaining presented variable intensity, with 35–90% labeled cells, the mean percentage value of 62.4±11.9 and the average composite score of 5.2. Negative cases belonged to the nodular, adenoid or morpheaform types. For the nodular type, the intensity of reactions was predominantly low, the number of labeled cells varied between 35–85% (mean value 57.7±10.9), and the average score was 3.2. By contrary, for the other two types, the intensity of reactions was predominantly moderate or high, with values between 45–85% (mean value 63±10.7) for the adenoid type and 65–90% (mean value 73.5±8.9) for the morpheaform type. Also, the average immunostaining scores for the adenoid and morpheaform types were higher compared with nodular type, respectively 6.5 and 8.3 (Table 3; Figure 1, D–F).

The HER3 immunoreactions in tumor cells had low intensity predominantly in the nodular and adenoid types compared to the morpheaform type, in which the intensity was moderate or high. Overall, the percentage range of labeled cells was wide, between 25–85%, with a mean value of 53.6±14 and an average composite score of 3.4. In case of nodular and adenoid types, the number of labeled

cells was 25–65% (mean value 48.6 ± 11.6) and 30–65% (mean value 50.8 ± 10.8), with an average scores of 2.4 and 2.8, respectively. For the morpheaform type, the values were significantly higher, respectively 65–85% labeled cells (71.8 ± 9.9 average) and an average score of 7.2 (Table 3; Figure 1, G–I).

The reactions of analyzed markers were observed mostly at the peripheral tumor islands, in the case of nodular type. In case of the adenoid type, the immunostainings were higher in the cells located in the inner cords of tumor compared with those in the periphery. In case of morpheaform type, there were no differences related to the signal reactions location in relation to the peripheral or central areas of the tumor islands.

In this study, we found no other statistical associations of EGFR, HER2 and HER3 immunostaining with clinical and histological analyzed parameters. Although the percentage values of the markers were higher in the advanced tumors, this appearance was not statistically significant ($p > 0.05$, *chi-square* test).

The statistical analysis revealed statistically significant higher values of EGFR and HER2 mean composite score values in morpheaform type ($p < 0.01$, *chi-square* test) and adenoid type ($p < 0.001$, *chi-square* test), compared with the nodular BCC type (Figure 2, A and B). Also, in case of HER3 mean composite scores, the values were

higher in morpheaform type compared with adenoid and nodular BCC types ($p < 0.001$, *chi-square* test) (Figure 2C).

The assessment of percentage values of analyzed markers indicated positive linear relation for the investigated BCC group, aspect which was statistically significant only in the case of EGFR/HER2 ($p < 0.05$, Pearson's test) (Figure 2D).

Discussions

In our study, BCCs were more common in men over 50 years old, most lesions being located in the head and neck regions. Also, the study indicated that nodular BCC with dimensions less than 2 cm, in stage I/II of disease were more frequent. The literature data indicate the BCC predominance in head and neck regions in 70–80% of cases, the risk for lesions development being of 100-fold higher in people over 55 years old [13]. Also, the risk of recurrence appears to be greater in tumors over 6 mm in size [12]. Most studies indicate an aggressivity and a lower recurrence rate for nodular and superficial subtypes compared with micronodular, infiltrative and morpheaform [2, 5, 12]. In this study were analyzed the nodular, adenoid and morpheaform subtypes of BCC, which are commonly encountered in clinical practice, most cases belonging to the nodular type.

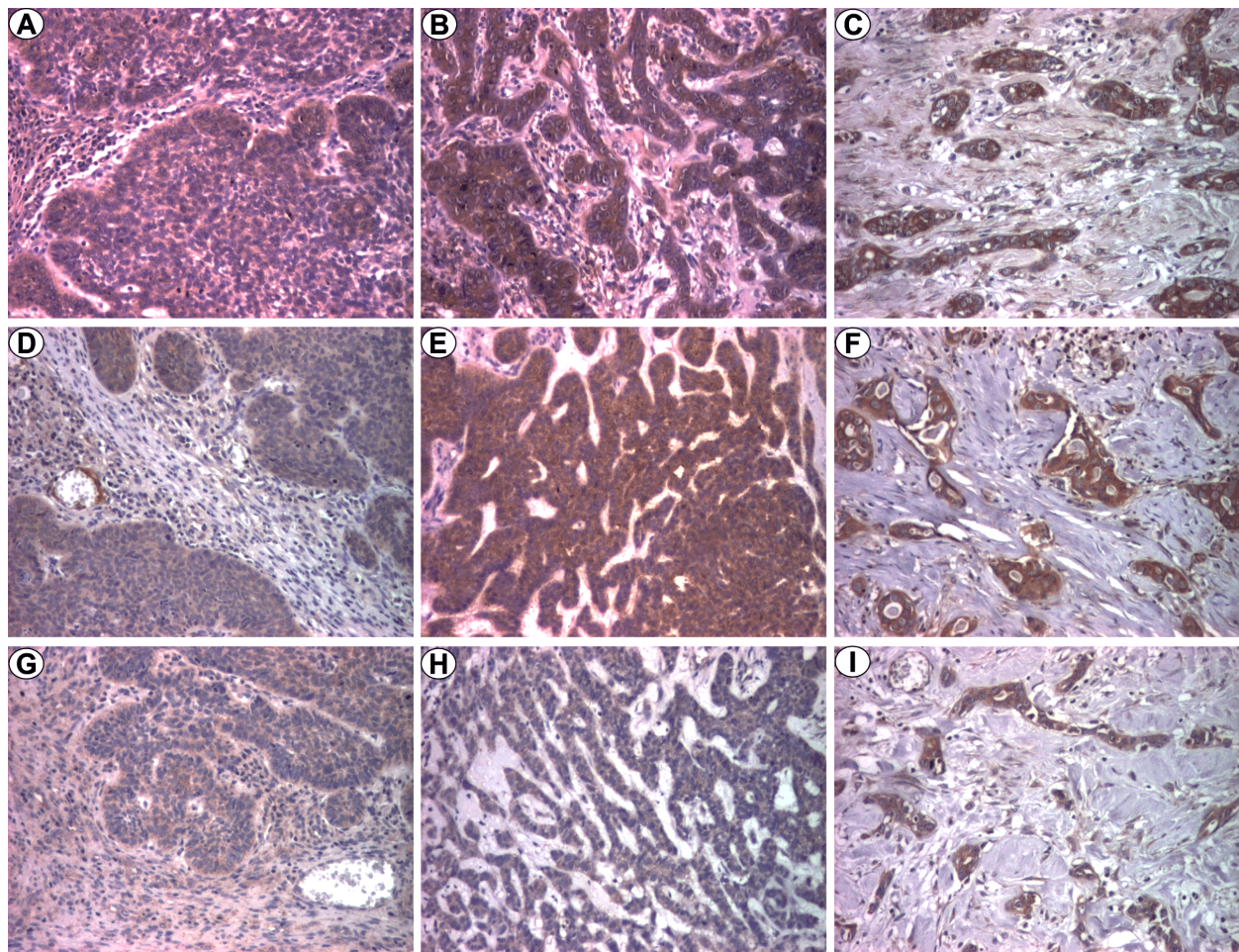


Figure 1 – (A, D and G) Nodular BCC; (B, E and H) Adenoid BCC; (C, F and I) Morpheaform BCC. EGFR immunostaining: (A–C) $\times 100$; HER2 immunostaining: (D–F) $\times 100$; HER3 immunostaining: (G–I) $\times 100$. BCC: Basal cell carcinoma; EGFR: Epidermal growth factor receptor; HER2: Human EGFR2; HER3: Human EGFR3.

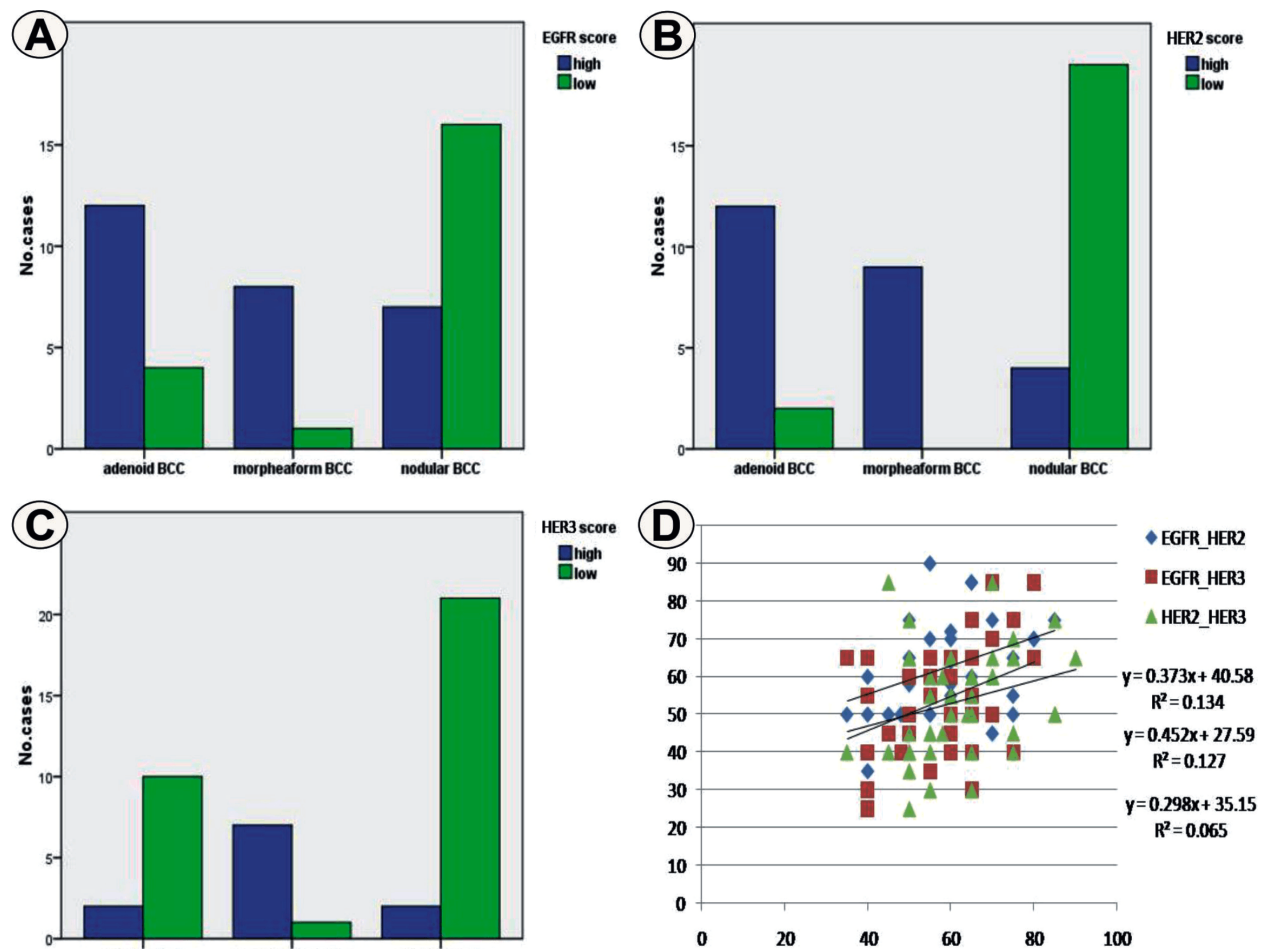


Figure 2 – (A) EGFR scores in relation to BCC types; (B) HER2 scores in relation to BCC types; (C) HER3 scores in relation to BCC types; (D) Distribution of mean values for EGFR, HER2 and HER3. BCC: Basal cell carcinoma; EGFR: Epidermal growth factor receptor; HER2: Human EGFR2; HER3: Human EGFR3.

The analysis of BCC histological differentiation in relation to the biomolecular mechanisms involved in the initiation and progression of these tumors is the subject of numerous studies that have attempted to exploit these relations therapeutically [6–8]. Nevertheless, the studies conducted to date about the EGFR expression in skin cancers and BCC are only few, and the obtained data are contradictory. EGFRs are involved in physiological and pathological processes, including inflammation and cancer pathogenesis and cooperation between these tyrosine kinase proteins appears to induce the initiation, proliferation and survival of the tumor [14–16]. The EGFRs analysis, particularly in case of HER2 and EGFR already targeted therapies for carcinomas with different locations (breast, lung).

In this study, most of the BCC analyzed revealed a triple positivity for EGFR, HER2 and HER3, the immunoreactions being identified both in tumor cells and adjacent normal skin. However, we found significant differences in the expression of markers in relation to the BCC subtype, in the sense of high immunostaining values in the morpheaform and adenoid types compared with the nodular type. Also, in this study we found a positive linear relation of the EGFRs immunoexpression.

EGFR activation can be done on alternative pathways, which involves the binding of specific ligands for EGFR

(epidermal growth factor, amphiregulin, transforming growth factor, heparin growth factor) and subsequently made homo- and heterodimerization with other members of the receptor family [17, 18]. The aspect suggests the importance of establishing a profile of EGFRs in evaluating their involvement in the processes of tumor initiation and progression. Thereby, in a study conducted by Krähn *et al.* on the expression of EGF receptors on normal tissues, BCC and squamous skin carcinomas revealed the isolated expression in the normal skin of HER2 or EGFR/HER2 and also of the triple expression of EGFR/HER2/HER3 more common in BCC and squamous carcinomas, underlining particularly that activation of HER3 is related to the appearance of the malignant phenotype [14]. In other studies that have attempted to characterize the cytokeratin profile of BCC to establish the origin of the lesion or in comparison to squamous cell carcinoma, the authors indicated the presence of EGFR expression in all cases analyzed, the immunostaining having high intensity, especially at the peripheral tumor islands [19, 20]. The involvement of EGFRs in BCC recurrence is outlined in studies such as one conducted by Yerebakan *et al.*, which indicated that Ki-67, CD31 and EGFR expressions are significantly higher in recurrent BCC compared with those without recurrences [21].

By comparison, there are studies that do not support

the EGFR differences expression in BCC comparing to normal skin, or their involvement in the pathogenesis of lesions. Thus, in the study conducted by Rittié *et al.*, the amounts of EGFR protein and mRNA were similar to normal skin and BCC and EGFR signaling activation was absent in BCC [22]. Other studies that analyzed HER2 expression in BCC and normal skin indicated the protein expression especially in the skin surface and annexes structures, and weak immunoreactions in the basal layer or BCC [23]. Furthermore, other authors argue the HER2 involvement in the pathogenesis of BCC, but in the condition of an expression significantly lower in tumor lesions compared to normal skin, suggesting the usefulness of HER2 in BCC diagnosis as a negative marker [24].

Although it is known the involvement of HER3 along with HER2 in the skin epithelial differentiation, the role of this protein in inducing tumor transformation at this level remains one less investigated. However, there are studies indicating the HER3 overexpression in cancerous lesions gastrointestinal, lung or breast, being involved in the tumor progression, aggressiveness and resistance to therapy [25–28]. In a recent study, which investigated the expression of HER3 in skin tumors, Wimmer *et al.*, indicated the expression of this protein in normal skin and also in investigated BCC, respectively an increased expression in these lesions in about 30% [10].

Overall, the studies conducted on EGFRs indicate their expression in case of normal skin and variable expression in BCC. These aspects may be due to relatively small groups of investigated patients, BCC subtypes introduced in these groups, and variables related to BCC location, stage or methods used for assessment. The expression of EGFRs in the normal skin can suggest their involvement in normal epithelial differentiation. The variable obtained immunostainings in this study, for different histopathological subtypes of BCC, can sustain the involvement of the EGFRs in BCC differentiation toward different growth patterns, essential aspect for lesions prognosis and recurrence.

✉ Conclusions

In this study, we found significant differences in the expression of EGFR, HER2 and HER3 in relation to the histological types of analyzed BCC. The morpheaform type was characterized by high scores of all EGFRs, the adenoid type by high values of EGFR and HER2, and the nodular type presented the lowest values, which designates the EGFRs as proteins involved in aggressiveness and histological differentiation of BCC. Further studies are needed to analyze the expression of EGFRs on all BCC histopathological variants and how the receptors interacts with the main biomolecular mechanisms involved in the progression of tumors.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper. All authors read and approved the final manuscript.

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