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Immunoexpression of p53 and COX-2 in basal cell carcinoma

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Abstract

Basal cell carcinoma (BCC) is a variety of cutaneous carcinoma associated with an excellent prognosis because it rarely metastasizes, but it can cause significant local destruction and morbidity if surgical excision is not made. In this study, we examined the immunohistochemical expression of p53 and cyclooxygenase-2 (COX-2) in 51 BCCs, nodular and infiltrative subtypes, with various Clark levels. The immuno-expression of p53 was identified in 74.5% BCC cases and COX-2 reactions in 88.2% of cases. The scores of p53 reactions revealed significant differences depending on Clark level and borderline significance with tumor type, the high positive scores being associated to infiltrative tumors and high Clark level. No differences were revealed between COX-2 scores with both Clark level and tumor type. The analysis of the percentage values of p53 and COX-2 indicated a positive linear correlation. The positivity of p53 and COX-2 in a large proportion of BCCs, regardless of histological type and of depth of invasion, supports the two markers involvement in tumor progression.

Keywords: basal cell carcinoma, Clark stage, p53, COX-2.

Introduction

Basal cell carcinoma (BCC) is one of the most common malignant tumors, characterized by a local infiltrative and destructive growth [1]. Although the etiopathogenesis of lesions is largely known, the BCC's diagnostic rate is steadily increasing, the risk of developing BCC being around 30% during lifetime [2].

One of the most affected genes in human tumorigenesis is p53, over 50% of cancers presenting mutations at this level [3, 4]. Ultraviolet (UV) radiation is the main risk factor for the development of skin cancers, with solid evidence between UV exposure and p53 mutations [5]. In cases of skin cancers induced by UV, some data indicated that p53 mutations represent an early genetic event in cutaneous carcinogenesis [5]. The proliferation of mutant p53 keratinocytes is the result of relative resistance of cells to apoptosis [6].

Cyclooxygenase-2 (COX-2) is a molecule with a possible role in cutaneous carcinogenesis, multiple studies indicated that tumor development and progression may have a substrate represented by high COX-2 expression in tumor cells, the aspect being related with the survival advantage [7]. The molecular complex substrate of COX-2 expression is not completely understood, but some evidence indicates that overexpression of this protein is associated with p53 mutations [8].

The aim of this study was to investigate p53 and COX-2 expressions in nodular and infiltrative BCC, with a variety of tumor depths.

Materials and Methods

The study included 51 cases of BCCs, from the Department of Dermatology and the Department of Plastic and Reconstructive Surgery of Emergency County Hospital of Craiova, Romania. The surgical specimens were fixed in 10% buffered formalin, processed by the classic paraffin embedding technique and Hematoxylin–Eosin (HE) staining. The classification of tumors was made according to the specialty literature recommendations [9, 10].

Subsequently, we performed serial sections which were processed for immunohistochemistry using a detection system based on amplification polymer [Histophine polymer–Horseradish peroxidase (HRP), Nichirei, Japan, ready to use, code 414151F]. In order to visualize the reactions, we used the 3,3'-diaminobenzidine (DAB) chromogen (code 3467, Dako) and for reactions validation, we used positive (colon adenocarcinoma) and negative (by omitting the primary antibody) external controls (Table 1).

Table 1 – *Panel with the antibodies used in the immunohistochemical study*

Antibody	Clone	Dilution	Antigen retrieval	External control
p53	DO-7	1:50	Tris-EDTA buffer, pH 9	Colon adenocarcinoma
COX-2	4H12	1:50	Citrate buffer, pH 9	Colon adenocarcinoma

COX-2: Cyclooxygenase-2; EDTA: Ethylenediaminetetraacetic acid.

We quantified the semiquantitative expression of p53 and COX-2, using a scoring system which has been assigned independent by two specialists, on the basis of staining intensity and percentage of positive cells.

Staining intensity was graded as 0 (no staining), 1 (low intensity), 2 (moderate intensity) and 3 (high intensity). The score of the percentage of positive cells was noted with 0 (<5% positive cells), 1 (6–25% positive cells), 2 (26–50% positive cells), 3 (51–75% positive cells) and 4 (>75% positive cells).

The multiplication of the intensity and percentage scores allowed the calculation of the final staining score (FSS): 0 (negative), low (1–4) and high (6–12).

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

Results

The present study involved 51 BCCs, of which 40 corresponding to the nodular type and 11 cases corresponding to the infiltrative type. According to the depth of the invasion, four cases were included in Clark II, 14 cases in Clark III, 31 cases in Clark IV and two cases in Clark V.

The immunostaining for p53 was observed in 38 (74.5%) cases, with nuclear pattern. The negative cases corresponded to nodular BCCs, the infiltrative forms being totally positive for this marker.

For the nodular type of BCC, the percentage of tumor cells stained was variable, the mean percentage of the marked cells being 55, 56.1±12.2, 84.7±5.7, and 77 for Clarks' level II, III, IV and V tumors, respectively. The intensity of reaction was constant high/moderate regardless the grade of invasion (Figure 1, A–D).
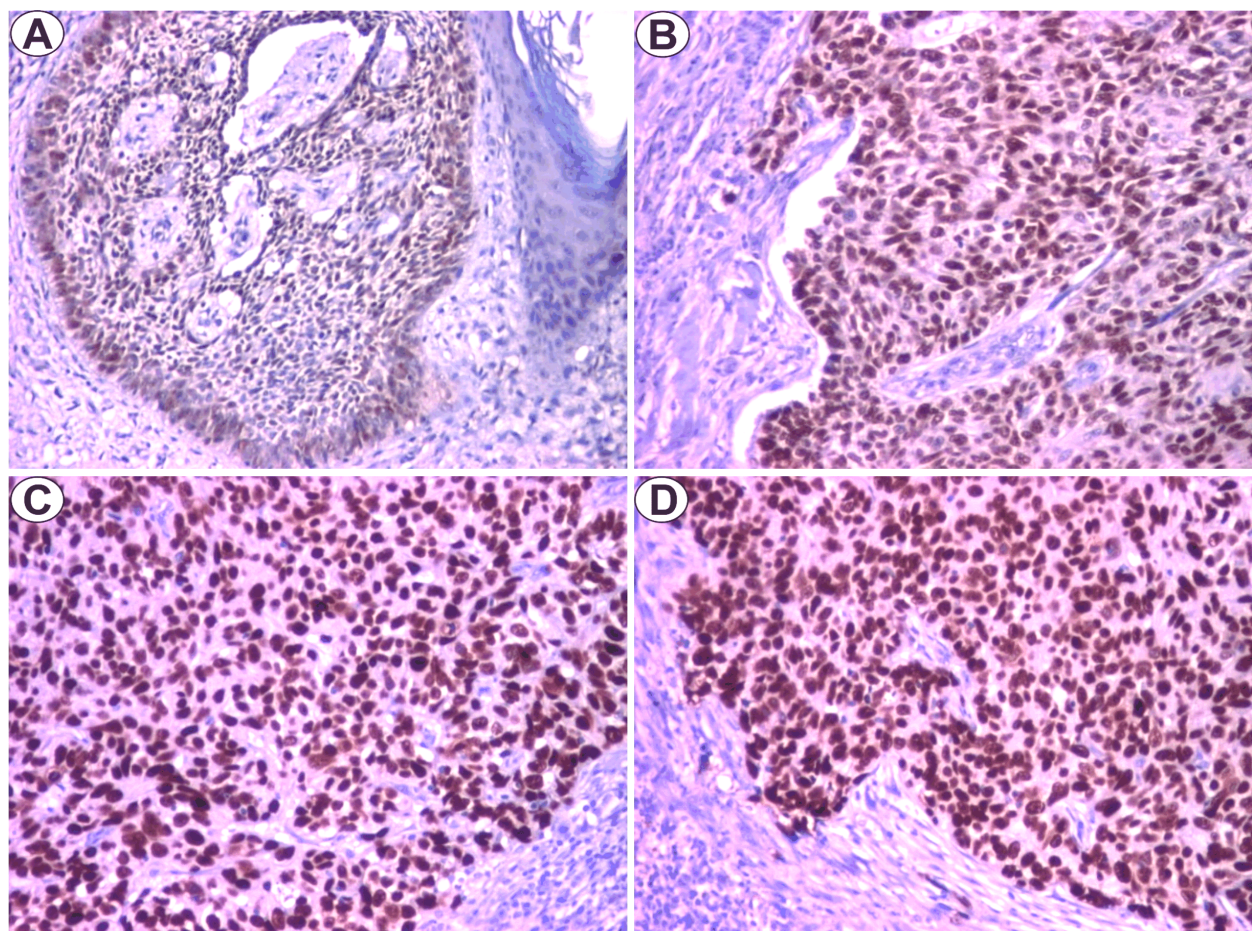

Figure 1 – *Nodular basal cell carcinoma: (A) Clark II; (B) Clark III; (C) Clark IV; (D) Clark V. Anti-p53 antibody immunostaining, ×100.*

The FSS values were 6 for Clark level II tumors, 7.1 for Clark level III tumors and 12 for Clark level IV and level V tumors (Table 2).

Regarding the infiltrative type of BCC, an average percentage of the marked cells was 77, 84.1±5.6, and 88 for Clark III, IV and V tumors, respectively. The intensity of the reaction was increased for all degrees of invasion. Thus, FSS values were 9 for Clark III tumors, 12 for Clark IV and V tumors (Figure 2, A–C).

COX-2 immunoreaction was observed in 45 (88.2%) of the investigated cases, with cytoplasmic diffuse pattern.

Negative cases corresponded to nodular BCCs, infiltrative forms being the same as for p53, all positive for this marker.

For the tumors with nodular pattern, the average percentage of marked cells progressively increased with the invasion depth (65, 73.5±11.8, 82.1±7.7 and 85) in Clark II, III, IV and V tumors, respectively. The intensity of the reaction was increased or moderate regardless of the depth of invasion (Figure 3, A–D). FSS values were 6.66 for Clark II tumors, 9 for Clark III tumors, 11 for Clark IV tumors and 12 for Clark V tumors.

For the infiltrative type of BCC, the average percentage of marked cells also increased with the invasion depth (80, 87.2±7.1 and 100 in Clark III, IV and V tumors, respectively). The intensity of the reaction was increased for all degrees of invasion (Figure 4, A–C). FSS values were 8 for Clark III tumors, 12 for Clark IV and Clark V tumors.

Table 2 – *Cases distribution depending on FSS average value*

Markers / Clark grade	Positive cases (No.) / FSS	p53		COX-2	
		Nodular	*Infiltrative*	*Nodular*	*Infiltrative*
Clark II	Positive cases (No.)	1	–	3	–
	FSS	6	–	6.66	–
Clark III	Positive cases (No.)	7	1	12	1
	FSS	7.1	9	9	8
Clark IV	Positive cases (No.)	18	9	18	9
	FSS	12	12	11	12
Clark V	Positive cases (No.)	1	1	1	1
	FSS	12	12	12	12

FSS: Final staining score; COX-2: Cyclooxygenase-2.

The statistical analysis indicated significant differences between p53 scores and Clark level ($p=0.008$) and a borderline statistical significance ($p=0.054$) with tumoral type, the high positive scores being associated to infiltrative tumors with a high Clark level (Figure 5, A and B). No statistical correlation was revealed between COX-2 immunoreactivity with both Clark ($p=0.328$) and tumor type ($p=0.696$). The analysis of the percentage values of p53 and COX-2 indicated a positive linear correlation ($p<0.001$) (Figure 5C).

⎙ Discussions

BCC originates from the basal cells of the epidermis and has an excellent prognosis because rarely metastasize, but if not excised it can cause significant local morbidity [11]. The only element of histological prognosis of biological behavior and therefore a major determinant of the therapeutic approach is the architectural pattern that causes indolent behavior (low-risk group) or aggressive (high-risk group) [12–16]. Indolent-growth subtypes include nodular and superficial BCC, and the aggressive growth tumors are infiltrating, metatypical and sclerosing BCC.

The analysis of p53 and COX-2 expression for the 51 cases of BCCs indicated a high percentage of tumor positivity for the two markers, regardless of the histological pattern or the depth of the invasion. Thus, p53 was positive in 74.5% of cases, and COX-2 was positive in 88%, which supports the two markers involvement in tumor progression. In various studies, COX-2 expression was also reported with increased incidence, ranging from 70–100% [8, 17, 18]. In addition, several studies have detected mutations of the *P53* gene in a fairly large proportion of BCCs, ranging from 38–100% [19–26].
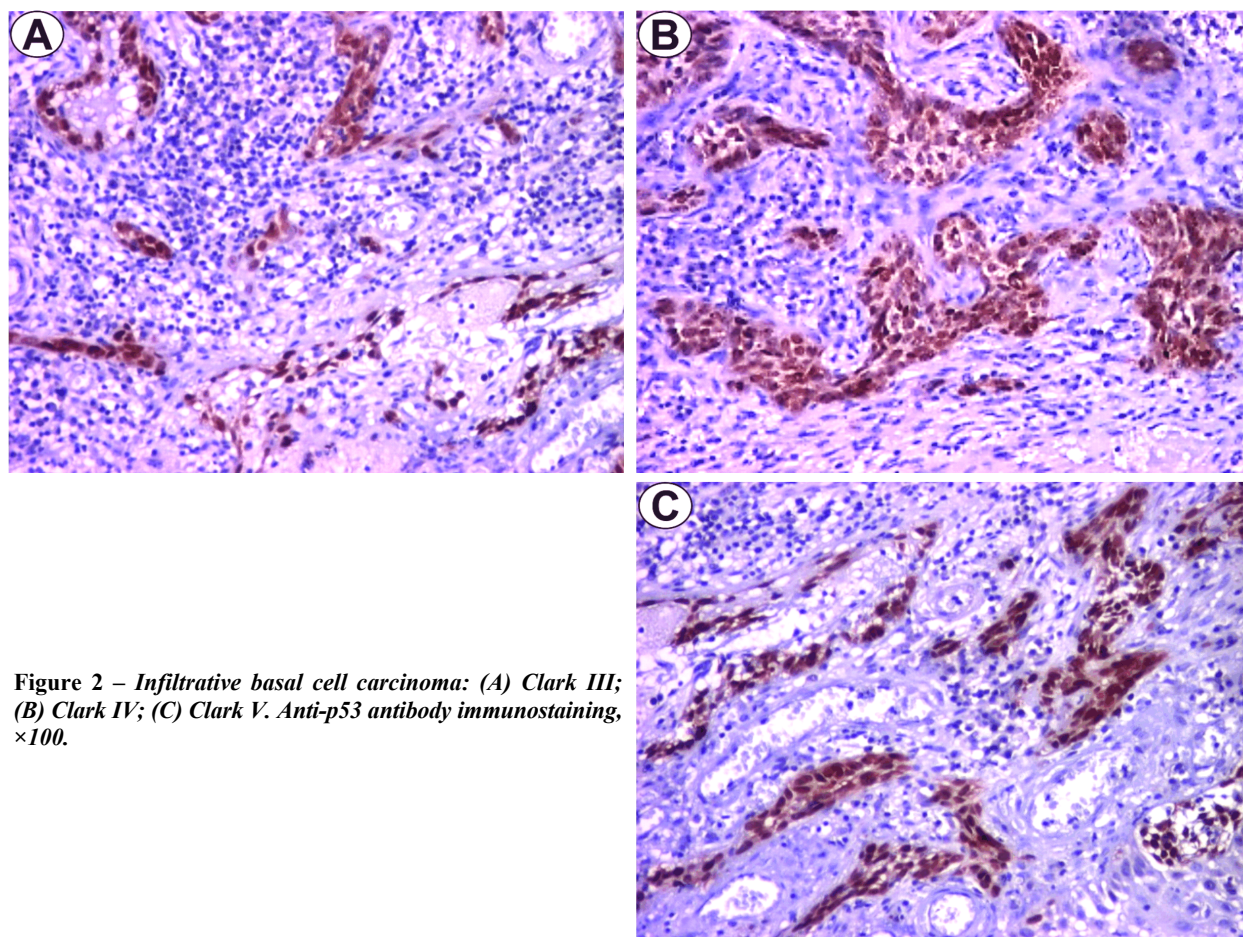
Figure 2 – *Infiltrative basal cell carcinoma: (A) Clark III; (B) Clark IV; (C) Clark V. Anti-p53 antibody immunostaining, ×100.*
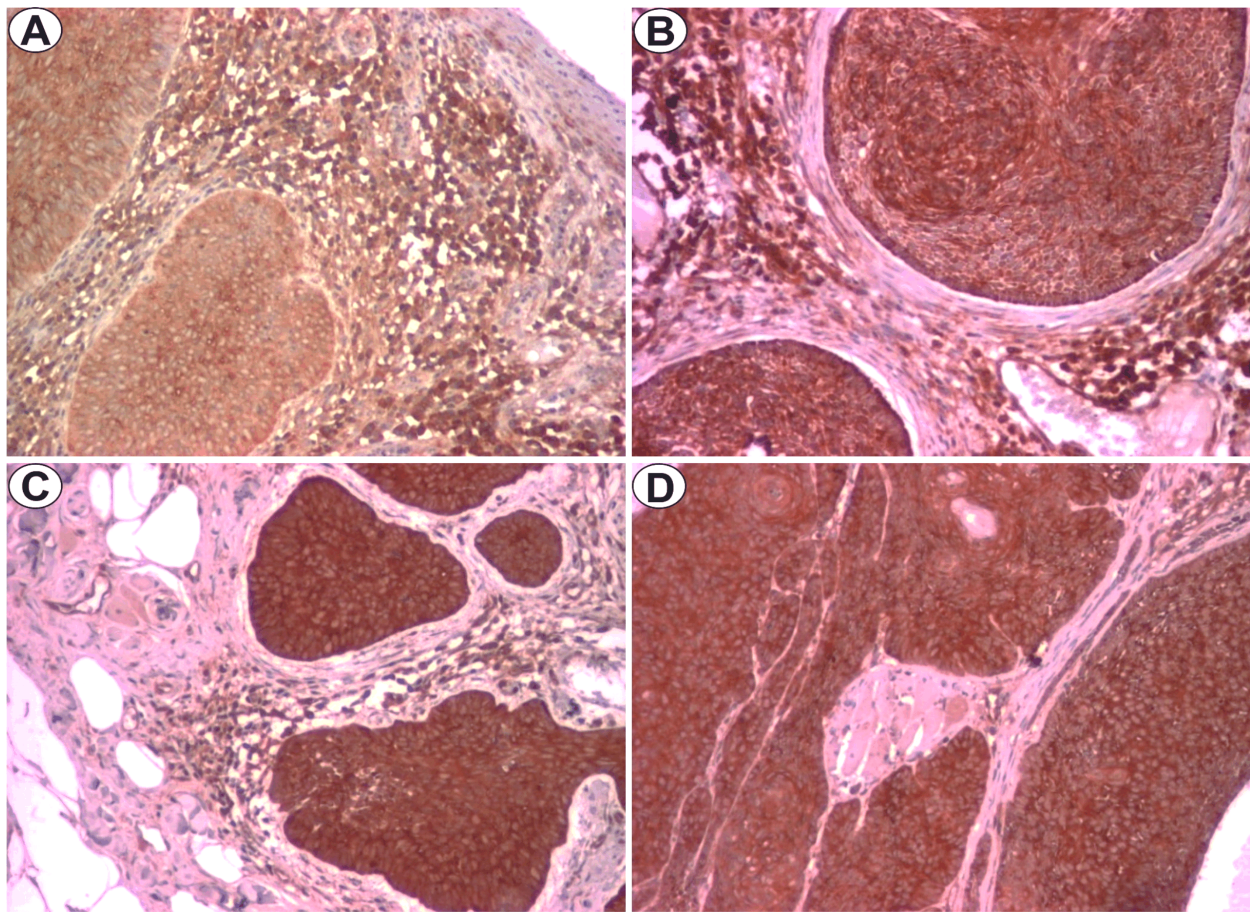
Figure 3 – *Nodular basal cell carcinoma: (A) Clark II; (B) Clark III; (C) Clark IV; (D) Clark V. Anti-COX-2 antibody immunostaining, ×100. COX-2: Cyclooxygenase-2.*
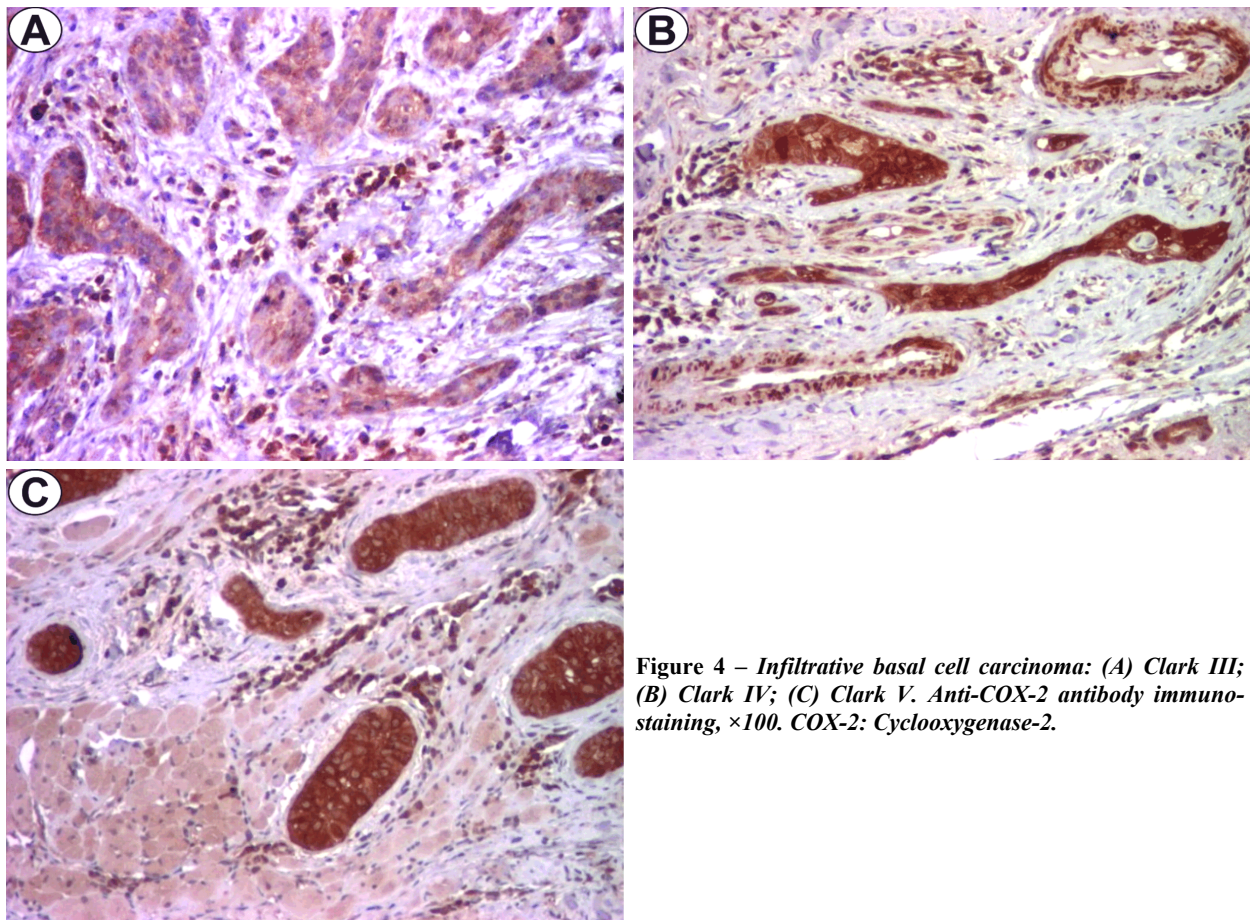

Figure 4 – *Infiltrative basal cell carcinoma: (A) Clark III; (B) Clark IV; (C) Clark V. Anti-COX-2 antibody immuno-staining, ×100. COX-2: Cyclooxygenase-2.*
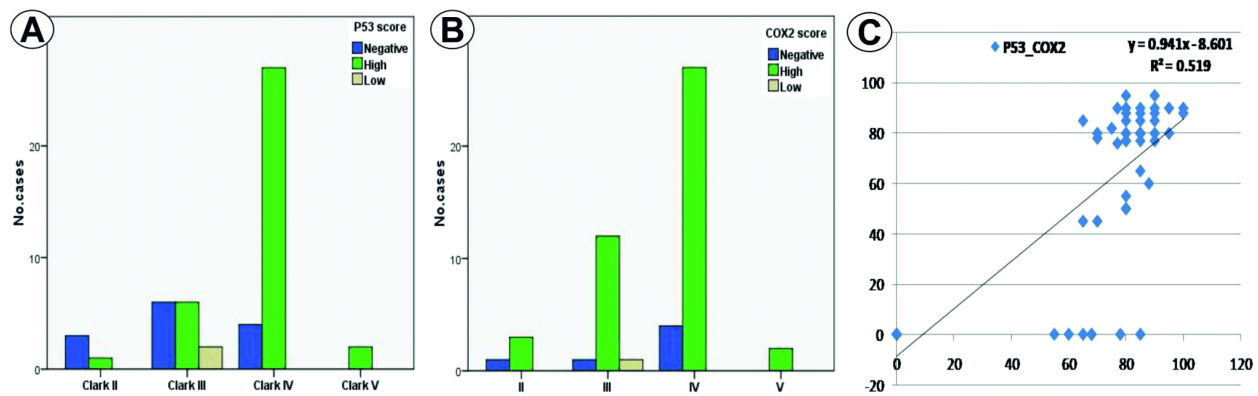
Figure 5 – *(A) Distribution of cases in relation with p53 scores and Clark level; (B) Distribution of cases in relation with COX-2 scores and Clark level; (C) Distribution of percentage values for p53 and COX-2. COX-2: Cyclooxygenase-2.*

The statistical analysis of p53 expression in the study revealed superior positive scores values in infiltrating tumors with an advanced Clark level. Several studies have studied the p53 expression in BCC variants with different degrees of aggressiveness and reported significantly greater expression of p53 in the aggressive group, claiming that p53 immunoexpression is an important prognostic factor for these tumors [27, 28]. However, other studies have found no any significant difference in p53 expression between aggressive and non-aggressive variants [18].

COX-2 is a biological marker with significant role in many neoplasias, including cutaneous carcinogenesis, where it acts by stimulating cell division, angiogenesis and inhibition of apoptosis [29, 30]. The presence of COX-2 was reported in normal skin, benign proliferative epidermis and skin malignant neoplasms [31–33].

In this study, the statistical analysis did not revealed any significant relation between COX-2 scores and tumor type or Clark level. One study reported that the degree of COX-2 expression is higher in tumors with deep invasion and it is also higher in the infiltrating BCC compared with the nodular one [34]. Another study indicated that COX-2 expression had higher values in infiltrating pattern of BCC compared with the nodular and superficial subtypes [17]. In contrast, other studies did not find any difference in COX-2 expression depending on the aggressiveness of the BCCs [18].

Although the results of studies that have focused on the expression of p53 and COX-2 are quite variable, it is considered that the increased expression of COX-2 in skin cancers is due to the p53 mutation [18, 35], COX-2 increasing p53 protein expression and suppressing apoptosis [36]. Other studies report that, although the COX-2 positivity rate in p53-positive skin tumors is high, the relation of the two proteins expression in skin cancers was not significant in the statistical analysis ($p > 0.05$) [8].

⌂ Conclusions

The positivity of p53 and COX-2 in a large proportion of BCCs, regardless of histological type and of depth of invasion, supports the two markers involvement in tumor progression. The positive linear relation between p53 and COX-2 may suggest a synchronous intervention in biomolecular mechanisms involved in the pathogenesis of BCCs.

Conflict of interests

The authors declare that they have no conflict of interests.

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