Assessment of the aggressive feature of basal cell carcinoma in the oral and maxillofacial region

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Summary

Background. **Basal cell carcinoma (BCC) is a very frequent skin malignancy. Due to its slow evolution and rare metastases it does not threat life. Still, certain locations and histological subtypes trigger a more aggressive character, which can only be revealed by specific immunohistochemical stains.**
Material and method. **37 archived formalin-fixed paraffin-embedded tissue samples of BCC from the oral and maxillofacial region were investigated by means of immunohistochemical (IHC) method, using the SABC (streptavidin biotin complex) indirect tristadial technique for the following markers: SMA (smooth muscle alpha actin), CD10, Ki67 and p53. Derived data have been statistically analyzed using the t-Student parametric test, paired two samples for mean variant, in MS-Excel 2003, running under Windows XP. Undetermined cases were eliminated.**
Results and discussions. **Most tumors proved to be histologically nodular (35.13%) and nodular infiltrative (32.43%) subtypes of BCC, the other 32.44% of cases being superficial, metatypical, morpheaform, keratotic, adenoid subtypes or with sebaceous and trichilemmal differentiation. SMA was positive in 33 cases (89.19%) in the tumor, in isolated cells, patches or diffuse in all tumor mass. The more aggressive BCC subtypes, such as nodular infiltrative and morpheaform evidenced zonal or diffuse positivity to SMA. Almost a quarter (8 cases - 24.25%) of SMA positive cases also revealed immunostaining to CD10 in tumoral cells. p53 had a mean of 17.22%, with a standard error of +/- 3.5%. The highest values (over 20%) were observed in nodular infiltrative and superficial BCCs. Proliferation marker Ki67 had a mean of 14.94%, with a standard error of +/- 1.8%.**
Conclusions. **Ki-67 and p53 proved to be independent prognostic and prediction markers, confirming the literature data. SMA diffuse positivity in tumoral cells and peritumoral stroma was useful in assessing the aggressive and invasive character of BCC. Although CD10 was positive in tumoral cells only in a quarter of the study cases, its immunoexpression was higher than that of SMA, thus proving to be a relatively sensitive marker, but less specific to BCC. The presence of CD10 in the invasion front underlines the aggressive character of nodular infiltrative BCC.**

Key words: **basal cell carcinoma, SMA, CD10, Ki67, p53.**

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Introduction

Basal cell carcinoma, the most frequent skin malignancy (65 to 75% of all skin cancers) arises from the basal cells of the epidermis and pilosebaceous units. It has a long evolution, slow growth, local destructive potential and rarely metastasizes. [1,2]. The clinical and microscopical features are polymorphous. The histological appearance includes undifferentiated (nodular and infiltrative subtypes) and differentiated forms. The differentiation is slight and made towards the cutaneous appendages of hair (keratotic BCC), sebaceous glands (BCC with sebaceous differentiation), apocrine/ eccrine glands (adenoid BCC). Many undifferentiated BCCs may show differentiation in certain areas, and most differentiated BCCs may contain areas lacking differentiation.

Differentiation is often directed toward more than one of the cutaneous appendages. There is no difference in the rate of growth between the two groups of BCCs. Other microscopic types are: morpheaform (sclerodermiform), fibroepitelioma, superficial, adamantinoid, granular, clear-cell and with matricial differentiation. [1,3,4]

Certain risk locations and histopathological subtypes may trigger deep invasion of tissue structures, with progression toward vital organs. This situation is detected in BCCs in the vicinity of face cavities (mouth, nose, ear, eyes) and in deeply penetrating BCC subtypes, such as infiltrative, morpheaform, micronodular or combination of these. [5].

Actin is largely responsible for cell motility and is only sparsely found in normal epithelial cells. An altered expression of actin in some malignancies may facilitate aggressive invasion. In BCC, beside the tumor cells, alpha-smooth muscle actin (SMA) can be identified in smooth muscle cells, myofibroblasts, pericytes, and myoepithelial cells. Myofibroblasts may appear in association with many reactive processes in various tissues and usually accompany malignant neoplasms. [6]

The CD10 antigen is a cell surface zinc-dependent metalloprotease that is expressed in a wide variety of cell types; in BCC, it is expressed by tumor cells and stromal cells. [7,8]

p53 is mostly expressed in aggressive BCC, and tumors that overexpress p53 have a poorer prognosis. [9] Several studies have shown that ultraviolet radiation is responsible for the induction of p53 mutations and perhaps for the initiation of both aggressive and nonaggressive BCCs. [10,11] The fact that both aggressive and nonaggressive BCCs as well as squamous cell carcinomas contained ultraviolet signature p53 mutations (C to T and CC to TT) at similar frequencies suggests that p53 mutations may play a role in tumor initiation but not in tumor progression. [10] Ki-67 antigen expression also differs between BCCs that recur and non-recurring BCCs. [12]

In order to assess BCC aggressive character, we performed immunohistochemical analysis of 37 formalin-fixed, paraffin-embedded tissue samples of BCC from the oral and maxillofacial region, by using the following IHC markers: CD10, smooth muscle actin, Ki67 and p53.

Material and method

Thirty-seven archived formalin-fixed, paraffin-embedded tissue samples of BCC, belonging to the Department of Pathology of Constanta Polyclinic no. 2, have been selected for the histopathological analysis, using the standard haematoxylin and eosin stain. Tumor biopsies are derived from the oral and maxillofacial region of 37 patients, who undergone surgery in the Constanta Oral and Maxillofacial Surgery Clinic for tumor excision.

A modified [13] IHC technique of Hsu S.M. et al. [14] was performed. Three μm thick sections from formalin-fixed paraffin-

embedded specimens were processed by indirect Streptavidin-Biotin-Complex method. Briefly, the procedure comprised: deparaffination in xylene and alcohol series, rehydration, washing in phosphate buffered-saline (PBS), blocking with normal serum, for 20 min, incubation with primary antibody overnight then with standard labeled streptavidin antibody biotin (LSAB kit, DAKO, Glostrup, Denmark); washing in carbonate buffer and developing in 3,3'-DAB hydrochloride/H_2O_2. All specimens were counterstained with Mayer's hematoxylin, examined and photographed on a Nikon Eclipse 600 microscope.

Negative control was made by using a primary irrelevant antibody or by replacing the secondary antibody with phosphate buffered-saline (PBS). Positive control was made comparatively with the expression of antibody investigated in the peritumoral cutaneous tissue (positive internal control on slides). Also, to ensure immunohistochemical accuracy, internal quality control was made, according to a quality guarantee certificate system (ISO 900 1/2001). The antibodies used in this study are presented in *Table 1*.

The distribution of SMA and CD10 positivity has been assessed using the modified Quick score method [15], which takes into account intensity and distribution of positivity: negative (no staining) = 0; weak (only visible at high magnification) = 1; moderate (readily visible at low magnification) = 2; strong (strikingly positive at low magnification) = 3. Ki-67 and p53 have been assessed semiquantitatively counting the number of stained nuclei per 100 tumor cells at high power field and expressed in percentage.

Data have been statistically analyzed using the Analysis Tool Pak of Microsoft Excel 2003, running under Windows XP Professional (descriptive statistical tests, t-Student parametrical test, "paired two sample for means" - "one group two-tails").

Results and discussions

The 37 tumor biopsies belonged to 35 patients, with the mean age of 62 years and a sex ratio: M/F=2/1. The histopathological subtypes of BCCs of the study batch are shown in Table 2.

Several nodular and nodular infiltrative BCCs, also showed areas of cystic transformation, melanin deposits, and adenoid and keratotic differentiation. Of the 37 BCC cases, 2 were relapses.

Table 2. Histopathological subtypes of basal cell carcinoma in the study batch

Histopathological subtypes	No. of cases
Nodular (N)	13 (35.13%)
Nodular infiltrative (NI)	12 (32.43%)
Morphea-like	1(2.70 %)
Sebaceous differentiation	1 (2.70%)
Keratotic	2 (5.40%)
Adenoid	1 (2.70%)
Trichilemmal differentiation	1 (2.70%)
Superficial	4 (10.81%)
Metatypical	2 (5.40 %)

We assessed the distribution of SMA and CD10 positivity in tumoral cells and in

Table 1. Antibodies used in the study

Antibody	Producer	Dilution	Clone	Specificity
CD10	Neomarkers	1:30	56C6	Stromal cells
SMA	Sigma	1:1500	1A4	Smooth muscle actin
Ki67	Dako	1:50	MIB 1	Proliferation index
P53	Neomarkers	1:50	DO7	Protein product of p53 gene

peritumoral stroma. Inconclusive cases were eliminated from the study.

SMA was positive in 33 cases (89.19%) in the tumor, in isolated cells, patches or diffuse in all tumor mass. The more aggressive BCC subtypes, such as nodular infiltrative and morpheaform displayed zonal or diffuse positivity to SMA. Stromal positivity was noticed in the cytoplasm of certain fusiform cells, with centrally-located oval nuclei, that morphologically resemble myofibroblasts, in 16 cases (43.24%), indicating the possibility of induction of myofibroblastic stromal changes in surrounding tissues by cytokines secreted from BCC cells. *(Figures 1 and 2)*

Our results are in accordance to those of Law et al. [16], who found that actin was present in the nodular component of 28% purely N-BCCs and 85% mixed NI-BCCs.

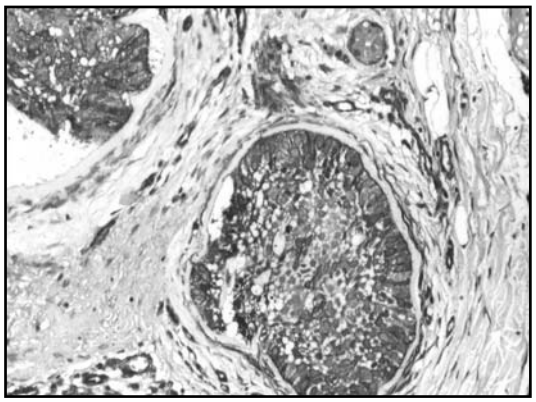

Figure 1. Positive IHC reaction to SMA in the tumor, peritumoral stroma and blood vessels, 10x

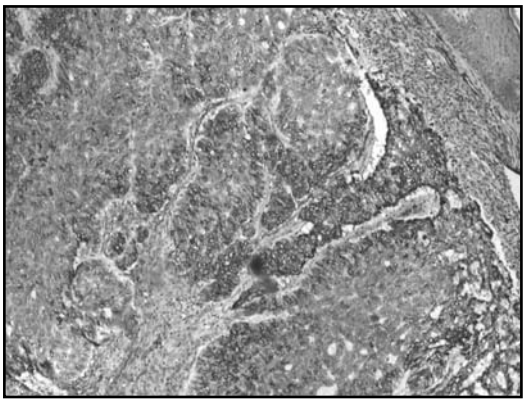

Figure 2. Positive IHC reaction to SMA in certain areas of the tumor, 4x

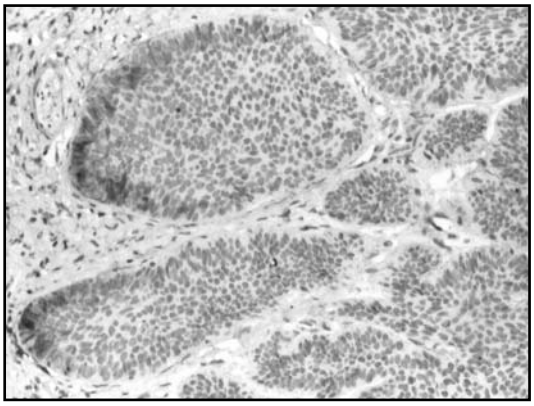

Figure 3. Positive IHC reaction to CD10 in the tumor invasion front, 10x

As for the presence of actin in peritumoral stroma, our results are comparable to those of Tsukamoto et al. [17], who found a positivity of 36%.

CD10 had diffuse citoplasmic positivity, in peripheral zones, in the invasion front *(Figure 3)*. CD10 was positive only in nodular infiltrative cases of BCC. Almost a quarter (8 cases - 24.25%) of SMA positive cases also showed immunostaining to CD10 in tumoral cells. Positivity to CD10 in peritumoral stromal cells was noticed in 8 cases (6 cases nodular infiltrative, 1 case superficial and 1 case of nodular type).

Our results differ from those of Yada et al. [18] and Pham et al. [19] who found a positivity of CD10 in BCC with values of 86% and 87% respectively.

In our study, p53 had a mean of 17.22%, with a standard error of +/- 3.5%. The highest values (over 20%) were noticed in nodular infiltrative and superficial BCCs *(Figure 4)*. The morpheaform BCC case in our study had low values, both for p53 and Ki67 (3-5%).

Other authors detected p53 in 83% of BCCs [9], 80% [20,21] or 48.7% [22].

Batinac Tanja et al. [21] state that accumulation of p53 mutations is age related. In our survey we found no difference between p53 expression in young patients as compared to old ones. p53 and Ki67 were positive only in nuclei of the basal layer, in cer-

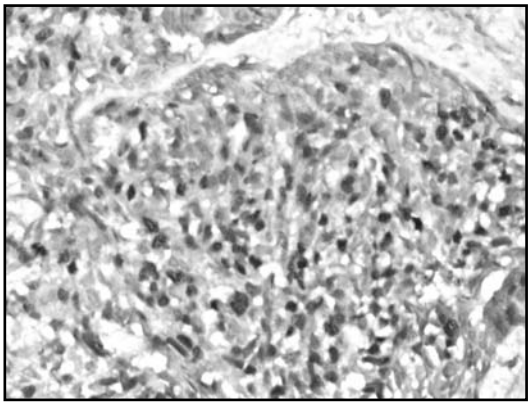

Figure 4. Positive IHC reaction to p53 in tumor cells nuclei, assessed as 30%, 20x

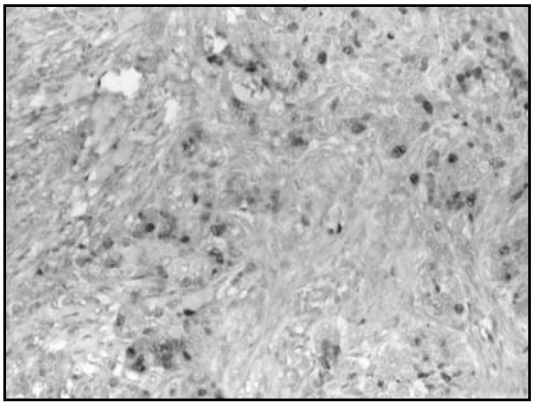

Figure 5. Positive IHC reaction to proliferation factor Ki67 in tumor cells nuclei, assessed as 15%, 10x

tain areas of the tumor overlying epidermis.

Proliferation marker Ki-67 had a mean of 15%, with a standard error of +/- 1.8% (Figure 5). Of the 33 cases of BCC positive to SMA in tumoral cells, 28 cases (84.84%) were also positive to Ki67; in the majority of situations (67%) the values registered were between 6 and 35%. In our study, there is no statistical correlation between p53 and Ki67, this certifying the fact that they are independent prognostic and prediction markers for BCC.

We also analyzed two cases of nodular infiltrative BCC relapses. Both showed immunostaining to SMA in tumor cells and peritumoral stroma. The positivity to CD10 was present only in the tumoral cells at the invasion front. The values of the proliferation markers were low (5-10%).

Conclusions

Ki-67 and p53 proved to be independent prognostic and prediction markers, bearing out the literature data.

SMA diffuse positivity in tumoral cells and peritumoral stroma was useful in assessing the aggressive and invasive character of BCC.

Although CD10 was positive in tumoral cells only in a quarter of the study cases, its immunoexpression was higher than that of SMA, thus proving to be a relatively sensitive marker, but less specific to BCC. The presence of CD10 at the invasion front underlines the aggressive character of nodular infiltrative BCC.

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