Cytokeratin expression in basal cell carcinomas of the oral and maxillofacial region

Albertine Leon [1], Zenaida Florea-Gr ãmadã [2], Mihai Ceau °u [3], Carmen Ardeleanu [4], Teofil Mehedinþi [5]

[1,5] Constanþa, [2,3,4] Bucharest, Romania

Summary

***Background*. Basal cell carcinoma (BCC) is one of the most frequent skin cancers, demonstrating polymorphous clinical and microscopical appearance, long evolution, slow growth and rather benign character. In spite of that, certain microscopical subtypes show aggressive character, with high local destructive potential. Diagnosis is easily made in general, but sometimes difficulties arise and specific methods are needed to point it out.**

***Material and method*.** 34 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 ABC (avidin biotin complex) indirect tristadial technique for three basal cytokeratin markers: 34BetaE12, Ber-EP4 and CK17. Obtained data were statistically analyzed using the t- Student parametric test, paired two samples for mean variant, in MS-Excel 2003, runnin gunder Windows XP. Undetermined cases were eliminated.

***Results and discussions*. Most of tumors proved to be histologically nodular (38.23%) and nodular infiltrative (32.35%) subtypes of BCC; 29.42% of cases were superficial, metatypical, morphea-like, keratotic, adenoid or sebaceous and trichilemmal differentiation subtypes. Antibodies revealed positive reactivity in 85.71% of cases for CK17, 83.33% for 34BetaE12 and 59.09% for Ber-EP4. A slightly direct positive correlation, statistically significant, was found between 34BetaE12 and Ber-EP4 (r = 0.32, p = 0.006) and between Ber-EP4 and CK17 (r = 0.4, p < 0.001); there was no correlation between 34BetaE12 and CK17 (independent markers).**

***Conclusions*. In our study, CK17 proved to be the most specific marker for BCC, in a strong direct proportion with Ber-EP4, the most sensitive marker.**

Keywords: basal cell carcinoma, skin cancer, cytokeratin, immunohistochemistry.

Introduction

Basal cell carcinoma is one of the most frequent malignant epidermal tumor. It arises from the basal cells of the epidermis and pilosebaceous units.

It is seen almost exclusively on the bearing skin, rarely on the palms, soles or mucous membranes [1]. In 80% of cases is located on the face and neck. It can appear on intact

1 Assistant Professor, DMD, Department of Pediatric Dentistry, Faculty of Dental Medicine and Pharmacy, "Ovidius" University of Constanta

2 - MD, MS, Resident in Pathology, "St. Pantelimon" Clinical Hospital, "Victor Babe °" National Institute of Pathology, Bucharest

3 - MD, PhD, Assistant Professor, Scientific Researcher, Department of Pathology, "Victor Babe °" National Institute of Pathology, "Carol Davila" University of Medicine and Pharmacy, Bucharest

4 - MD, PhD, Senior Researcher, Professor of Pathology, Head of the Department of Pathology, "Victor Babe °" National Institute of Pathology, "Carol Davila" University of Medicine and Pharmacy, Bucharest

5 - MD, PhD, Professor, Department of Histology, Faculty of Medicine, "Ovidius" University of Constanta

skin or it can develop on premalignant lesions (in approximately 50% of cases). [2]

The maximum of incidence is seen in old adults (60-80 years old), but the frequency is increasing in young adults, too (under 40 y.o.) The incidence is between 500 and 1,000 at 100,000 inhabitants, even higher in sunny regions; 10,000 new cases are recorded yearly in Romania. [2]

From **histological** point of view, BCC can be divided into two groups; ***undifferentiated*** (*solid* type, with *circumscribed* (*nodular*) and *infiltrative* subtypes) and ***differentiated***. 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). Both of them are sharing common features: many undifferentiated BCC may show differentiation in some areas, and most differentiated BCC may contain areas lacking differentiation.Often, differentiation is directed toward more than one of the cutaneous appendages. A BCC may show areas of pilar, sebaceous and adenoid differentiation, mixed with solid areas. There is no difference in the rate of growth between the two groups of BCCs. [1]

The *solid* subtype consists of nests of basal cells, showing prominent palisading of the peripheral layer, surrounded by a typical loose stroma, which contains myofibroblasts and often exhibits mucinous change. Cleft-like retraction spaces, some of artifactual nature and others resulting from the accumulation of stromal mucin, are often seen between the epithelial nests and the stroma. Melanin can accumulate in dermal macrophages between the tumor nests and result in a "pigmented" appearance clinically. Intercellular amyloid material is not infrequent, sometimes accompanied by the deposition of immunoglobulins. Tumor necrosis and cellular dyshesion result in the formation of cystic spaces. Mitotic activity (sometimes accompanied by atypical forms), marked atypia with appearance of bizarre ("monster") tumor cells, and giant cell formation may occur, but there is no convincing evidence that any of these features carries prognostic significance. [3]

The *infiltrative* subtype consists of elongated strands, only a few layers thick and with little or no palisading of the peripheral cells. The strands invade deeply into the dermis; sometimes they reach the muscles, cartilage, or even the bone. This type is an aggressive one.

Other microscopic types are: *morpheaform, fibroepithelioma*, *superficial*, *adamantinoid*, *granular*, *clear-cell* and with *matricial differentiation*. [1]

Diagnosis can be easily made by histopathological examination, but sometimes errors occur, especially when trying to differentiate BCC from certain squamous basal cell carcinoma, metatypical carcinoma, tricoepithelioma, desmoplastic epithelioma. In such cases immunohistochemical stains are used. Immunohistochemistry (IHC) combines anatomical, immunological and biochemical techniques for the identification of specific tissue components by means of a specific antigen/antibody reaction tagged with a visible label. IHC makes it possible to visualize the distribution and localization of specific cellular components within a cell or tissue.

Cytokeratins (CK) represent the most fundamental markers of epithelial differentiation. Not all keratins are synthesized simultaneously by any one cell; different subsets of keratins are expressed in different stages of development, during the course of terminal differentiation, and in different epithelia. Thus, all epithelia can be classified based upon CK protein expression.

When an epithelium undergoes malignant transformation, usually its CK profile remains constant. Thus, by help of CK expression, various carcinomas can be detected. [4]

Material and method

Thirty-four 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 34 patients who undergone surgery in the Constanta Oral and Maxillofacial Surgery Clinic for tumor excision.

The IHC technique, developed by Hsu S.M. et al. [5] is an indirect tristadial method, based on the initial technique of unlabeled antibody introduced for the first time by Sternberger in 1970 [6].

Immunohistochemistry was performed on 3 μm thick sections from formalin-fixed paraffin-embedded specimens, according to the Avidin-Biotin-Complex method of Hsu [5], modified by Bussolati and Gugliotta [7]. 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 CK positivity was assessed semiquantitatively using the modified Quick score method [11], 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.

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"), correlation and regression tests were used.

Table 1. Antibodies used in the study

Antibody	Producer	Dilution	Clone	Specificity
34BetaE12	DAKO	1:50	Polyclonal	CKs 1 and 10 (suprabasal layers)CKs 5 and 14 (basal layer) [4,8,9]
Ber-EP4	DAKO	1:200	Polyclonal	Two glycoproteins, 34 and 38 kDa, located on the basal cell membrane surface [10]
CK17	DAKO	1:40	E3	Basal cells

Results and discussions

The 34 tumor biopsies belonged to 34 patients, with a mean age of 63.23 years. The histopathological subtypes of BCCs of the study batch are shown in *Table 2*.

Most tumors proved to be histologically nodular (38.23%) (*Figure 1*) and nodular infiltrative (32.35%) subtypes of BCC.

Several nodular and nodular infiltrative BCCs also showed areas of cystic transformation (*Figure 2*), melanin deposits, and adenoid and keratotic differentiation. From the 34 BCC cases, 2 were relapses.

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

Histopathological subtypes	No. of cases
Nodular	13 (38.23%)
Nodular infiltrative	11 (32.35%)
Morphea-like	1 (2.94%)
Sebaceous differentiation	1 (2.94%)
Keratotic	2 (5.88%)
Adenoid	1 (2.94%)
Trichilemmal differentiation	1 (2.94%)
Superficial	3 (8.82%)
Metatypical	1 (2.94%)

Figure 1. BCC, nodular type, HE, 10x

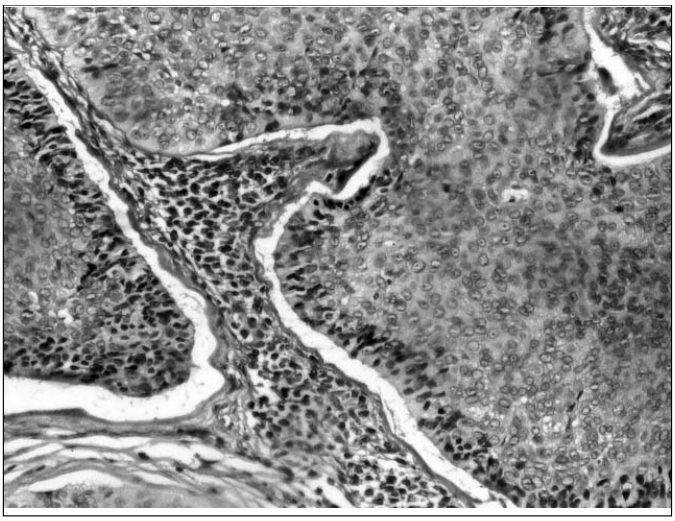
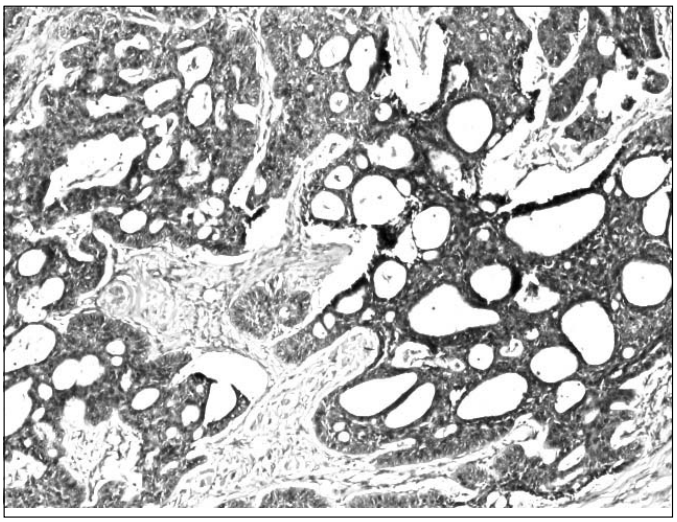


Figure 2. BCC showing cystic degeneration, HE, 4x



Cytokeratins expression in BCC has been analyzed, by using polyclonal antibodies: Ber-EP4 (22 cases - 64.7%) and 34BetaE12 (18 cases - 52.94%) and monoclonal antibodies - CK17 (28 cases - 82.35%). Inconclusive cases have been eliminated. In certain cases, two antibodies have been used for the same case.

First, we have tested the positivity to *BerEP4*, considered as the most specific marker for BCC [12,13,14,15,16], aiming to determine its distribution in BCC types. We tested it on a number of 22 cases of BCC. Data obtained are not in accordance with the literature; strong positivity was recorded only in two cases; 6 cases showed moderate positivity (27.27%) (

showed weak positivity (22.7%) and 9 cases were negative (40.9%). The tumor overlying epidermis showed negative reaction.

We studied the expression of *34BetaE12* in 18 cases, out of which 10 showed strong positivity (55.5%) (*Figure 4*), 5 cases showed moderate positivity (27.7%) and 3 cases were negative (16.66%). The overlying epidermis showed diffuse positivity to 34BetaE12. Though the sensitivity of 34BetaE12 is higher than that of BerEP4, its specificity in differentiating BCC form other tumors arising from the squamous epithelium is reduced; such tumors as trichoepithelioma, seborrheic keratosis and even squamous cell carcinoma are positive for this antibody.

Figure 3. Positive IHC reaction for Ber-EP4 in nodular BCC, 20x

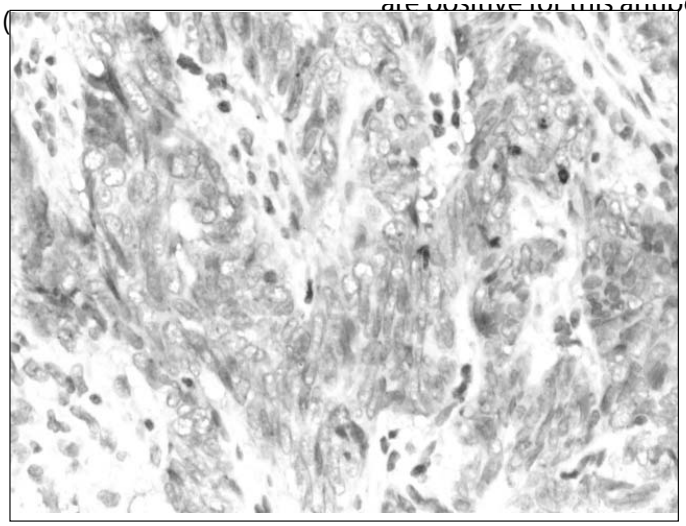
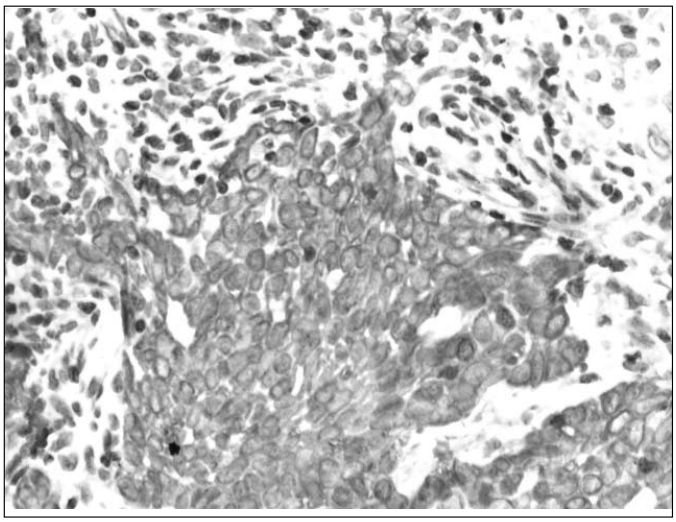


Figure 4. Strong positive reaction for 34BetaE12 in nodular BCC, 20x



The expression of *CK17* was studied in 28 cases; diffuse (strong) positivity was noticed in 18 cases (64.28%) (*Figures 5, 6*); in 6 cases (21.43%) the positivity was moderate and 4 cases (14.28%) were negative. Regarding CK17, our results show that most of the cases (24 out of 28) stained positively (strong and moderate), with only 4 cases of negative staining; these results are similar to those of Apaydin et al. [17], who found 17
out of 20 BCCs to stain positively to CK17 and of Kurtzen H et al. [18], who found an homogenous expression of CK17 in BCC.

Figure 5. Positive IHC reaction for CK17 in nodular BCC, 20x

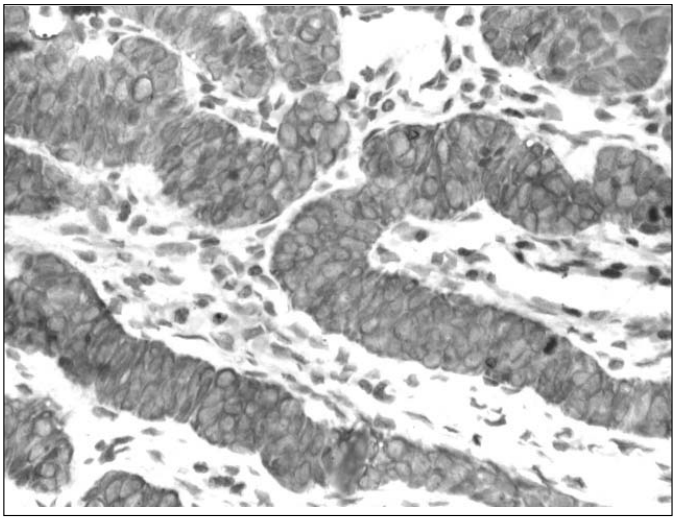
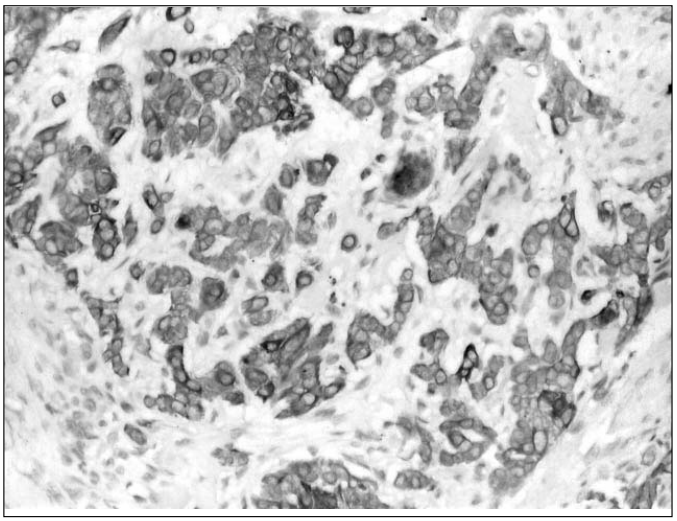


Figure 6. Positive IHC reaction for CK17 in infiltrative BCC, 20x



The comparative analysis of the three antibodies resulted in the following correla-
tions1. Between 34 Beta E12 and Ber-EP4 there is a weak direct correlation, statistically significant (r = 0.32, p = 0.006), *Chart 1*.

2. Between Ber-EP4 and CK 17 there is a strong direct, positive correlation, statistically significant (r = 0.4, p <0.001), *Chart 2*.

3. No correlation has been found between 34 Beta E12 and CK 17, the two variables being independent.

Chart 1. Correlation between 34BetaE12 and Ber-EP4 in BCCs of the study batch

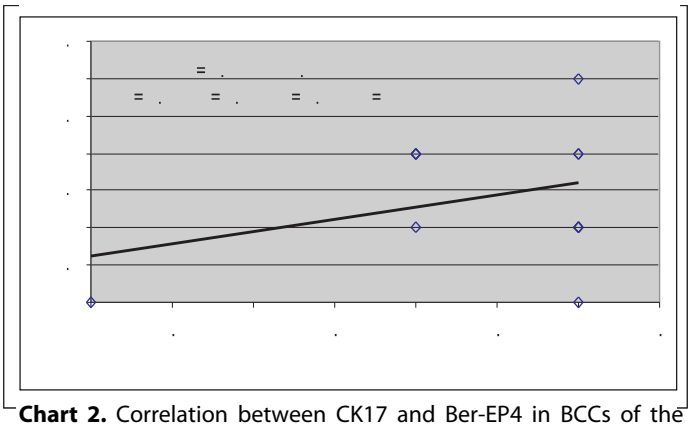
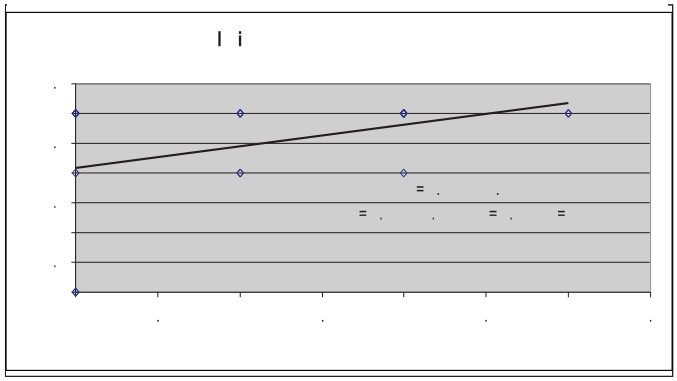


Chart 2. Correlation between CK17 and Ber-EP4 in BCCs of the study batch



Conclusions

The cytological markers we assessed by help of immunohistochemical methods, proved themselves useful in the detection and diagnosis of BCCs, fact confirmed by our results, which showed intense positive staining in the majority of cases. In our study, CK 17 proved to be the most specific marker for BCC, in a strong direct proportion with Ber-EP4, the most sensitive marker. These markers, expressed by basal cells and the suprabasal layer confirm the diagnosis of BCC, tumor that is derived from the basal cells of epidermis and skin appendages. Immunophenotypical variability of cytokeratins in BCC proves the heterogeneity of their expression in basal-type cells.

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Correspondence to: Dr. Albertine Leon, DMD, Assist. Prof., Faculty of Dental Medicine and Pharmacy, "Ovidius" University of Constanta, Romania. 7, Ilarie Voronca str., 900684 Constanta, Romania. E-mail: dr_aleon@yahoo.com