

Cytokeratin 17, Cytokeratin 19 and BerEP4 expression in basal cell carcinoma and adjacent epithelial structures of the skin

Vladimír Bartoš

Martin's Biopsy Center, Ltd., Martin, Slovakia.

Abstract

Background Cytokeratin (CK) gene expression in the skin reflects the type of epithelium, the differentiation state of epithelial cells and is subjected to striking modulation upon various challenges including carcinogenesis.

Objective The aim of the study was to evaluate an immunohistochemical expression status of CK17, CK19 and epithelial cell adhesion molecule EpCAM (BerEP4) in basal cell carcinoma (BCC) and adjacent epithelial structures of the skin.

Material and Methods Biopsy samples from 20 cases of BCC were investigated. All were stained for CK17 (clone E3), CK19 (clone RCK108) and EpCAM (clone BerEP4) by immunohistochemistry.

Results In normal skin, all three markers were constantly positive in the hair follicle components. They were typically negative in the interfollicular epidermis, but the epidermis overlying the tumor and at the edges of tumor mass expressed CK17 in the vast majority of cases. In BCC tissue, the CK17, CK19 and BerEP4 were positive in 100% (20/20), 40% (8/20), and 100% (20/20) of the cases.

Conclusion BCC constantly express CK17 and BerEP4 and is variably positive for CK19. Since all three markers are strongly positive in the hair follicle but not in the interfollicular epidermis, the staining pattern of CK17, CK19 and BerEP4 in BCC may provide an evidence to the suggestion that the tumor differentiation is toward follicular germinative epithelium.

Key words

Basal cell carcinoma, hair follicle, CK17, CK19, BerEP4.

Introduction

Cytokeratins (CK) represent a large family of intermediate filament proteins. They are considered a specific marker of epithelial cells.¹⁻³ More than 20 epithelial cytokeratins have been identified until now that are divided by molecular weight and isoelectric points into two types.¹⁻³ The type I cytokeratins have a relatively

low molecular weight and numerically include CK10–CK20.¹⁻³ The type II cytokeratins possess a higher molecular weight and numerically include CK1 – CK9.¹⁻³ The cytokeratins exhibit a high degree of tissue specificity. In the skin, CK gene expression reflects the type of epithelium (e.g. epidermis vs. pilosebaceous unit), the differentiation state of epithelial cells and is subjected to striking modulation upon various challenges including carcinogenesis.² Among a broad variety of cytokeratins, the CK17 and CK19 belong to the acidic type I category. In mammalian skin, both are

Address for correspondence

Dr. Vladimír Bartoš, PhD., MPH
Martin's Biopsy Center, Ltd.
Prieložtek 1, Martin, 036 01, Slovakia.
E-mail: vladim.bartos@gmail.com

principally synthesized in the appendages.² BerEP4 (epithelial cell adhesion molecule, EpCAM) is another intracytoplasmic/ membrane glycoprotein found in the vast majority of epithelial cells, with the exception of squamous epithelium and mesothelium. The goal of this study was to evaluate an immunohistochemical expression status of CK17, CK19 and BerEP4 in BCC tissue and adjacent cutaneous epithelial structures.

Material and Methods

Representative biopsy samples from 20 cases of primary BCC of the skin (12 being of the nodular subtype, 6 nodular-infiltrative and 2 infiltrative BCCs) were enrolled into this study. They were obtained from 19 patients (10 males, 9 females) in the age range of 37-88 years (mean age 73.9 y.). Topographic locations of the lesions were as follows: the head (16 cases), the upper/lower extremities (3 cases), and the back (one case). Biopsy samples were routinely processed and all were simultaneously stained for CK17 (monoclonal mouse antihuman antibody, clone E3, DAKO, ready-to-use), CK19 (monoclonal mouse antihuman antibody, clone RCK108, DAKO, ready-to-use), and EpCAM (monoclonal mouse antihuman antibody, clone BerEP4, DAKO, ready-to-use) according to manufacturer's instructions. Cytoplasmic and membranous expression of the markers was considered positive immunostaining. The results were interpreted descriptively, because the small number of the cases investigated did not allow a statistical evaluation.

Results

Cytokeratin 17

In normal skin, CK17 showed a strong immunoreactivity in the outer root sheath of hair follicles (**Figure 1**). Further, there was found a

mild to moderate positivity in the duct and secretory portions in most sweat and sebaceous glands, although a minority of them were completely negative. CK17 was not normally expressed in the epidermis. All BCCs (20/20; 100%) showed a diffuse intense positivity for CK17 regardless of histologic subtype. While normal epidermis distant from the tumor was constantly CK17-negative, the epidermis overlying the tumor (**Figure 2**) and at the edges of the tumor mass apparently expressed CK17 in the vast majority of cases.

Cytokeratin 19

As for CK19, the hair bulbs and the outer root

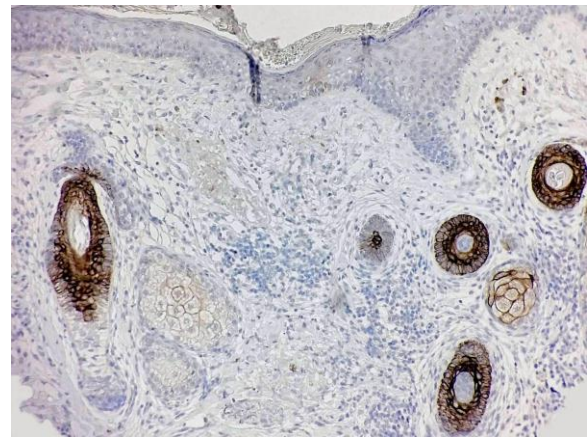


Figure 1 Strong CK17 immunoreactivity in the outer root sheath of hair follicles. Normal epidermis is negative (magnification 100x).

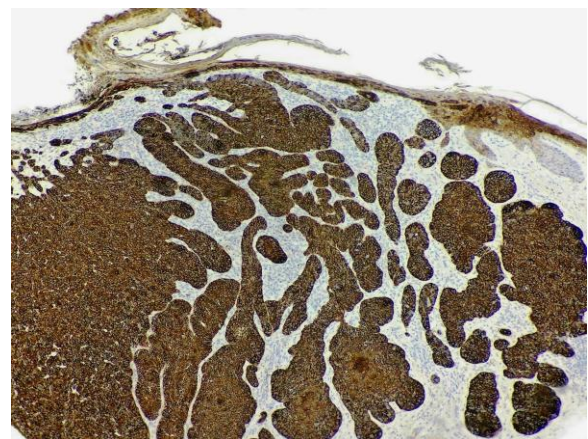


Figure 2 Diffuse intense CK17 expression in the BCC tissue. Epidermis overlying the tumor is obviously reactive (magnification 20x).

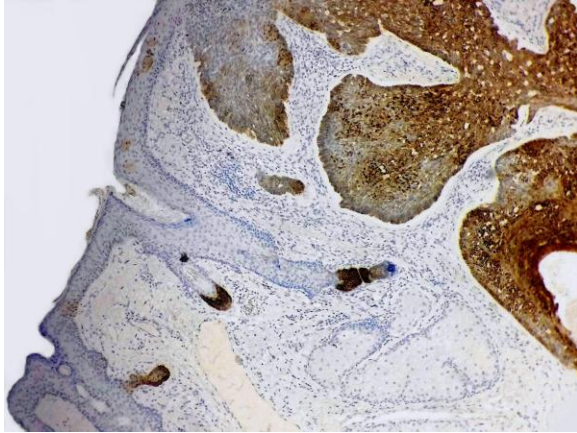


Figure 3 Uneven CK19 expression in the BCC tissue. There is a strong positivity in the hair bulbs, but isthmus, infundibulum and epidermis are negative (magnification 40x).

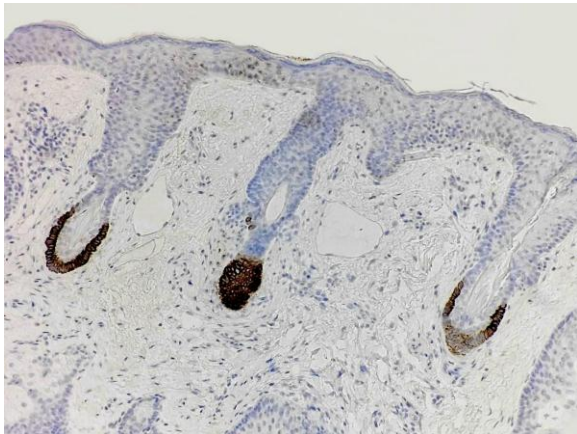


Figure 4 Strong BerEP4 immunoreactivity in the hair bulbs. Isthmus, infundibulum and epidermis are negative (magnification 100x).

sheath of hair follicles exhibited a constant strong positivity. In addition, there was recorded a moderate to strong reactivity in the duct and secretory portions in most sweat glands. Epidermis and sebaceous glands were negative. Focal moderate to strong reactivity for CK19 was found in eight BCCs (40%) (**Figure 3**). They included six nodular and two nodular-infiltrative subtypes and all but one were located on the head.

BerEP4

BerEP4 reacted positively with the follicular germinative cells at the lower end of catagen

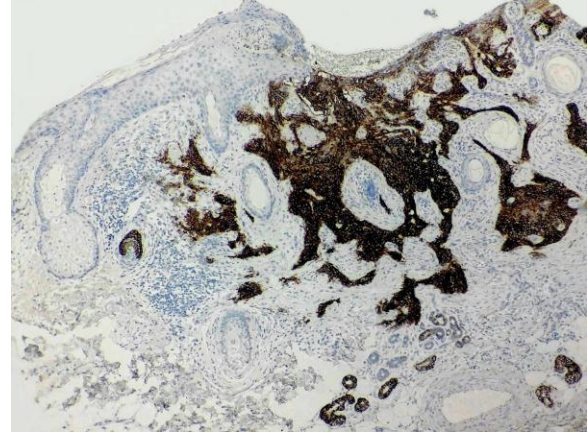


Figure 5 Diffuse intense BerEP4 expression in the BCC tissue. Adjacent epidermis is negative (magnification 20x).

hair bulbs (**Figure 4**) and secretory portions of eccrine glands. BerEP4 was not expressed in the epidermis and sebaceous glands. All the cases studied (20/20; 100%) revealed a diffuse cytoplasmic and membranous positivity for BerEP4 throughout the whole lesion (**Figure 5**).

Discussion

BCC represents the most common cutaneous malignancy in white-skinned people.⁴ In Asian population, it has been found the most prevalent skin cancer in Pakistan⁵ but the second most frequent skin malignancy in China,⁶ following squamous cell carcinoma. Although a cellular origin of BCC is not clearly defined, some studies have indicated^{7,8} that it arises from pluripotent stem cells within hair follicle. In the current study, the author has investigated an expression of CK17, CK19 and BerEP4 by immunohistochemistry in a series of 20 BCCs. It was found that BerEP4 was strongly positive in all BCCs, irrespective of histological subtypes and topographic locations. This is in line with the results of many researches done until now. All but two studies have shown⁹⁻¹³ that this marker was highly expressed in all BCCs analyzed. The exceptions are the papers published by Japanese¹⁴ and Iranian¹⁵ authors. In the study of Ansai *et al.*¹⁴ among 31 BCCs a

single case (morpheic subtype) was negative for BerEP4. In the analysis of Rajabi *et al.*¹⁵ out of 40 tumors three cases (adenoid, nodular, and infiltrative subtype) were BerEP4-negative. Further, it has been demonstrated that CK17 showed a diffuse strong staining in all analyzed tumors, without differences between the subtypes neither in the intensity nor in the extent. This also corroborates the results of other authors.^{13,16,17} However, an interesting finding was a CK17-immunoreactivity in intact epidermis situated in the vicinity of tumor tissue. Under physiological circumstances, the human interfollicular epidermis (apart from palmoplantar location) does not express CK17. While basal keratinocytes particularly express CK5 and CK14,^{2,18} more differentiated keratinocytes in the suprabasal layers downregulate them and start to produce CK1 and CK10.^{2,18} However, upon pathologic conditions, irritated keratinocytes induce de novo transcription of CK6, CK16, and CK17 which are normally not expressed in the interfollicular epidermis.^{2,18} These cytokeratins have been recognized as barrier alarmins and collectively provide a flexible scaffold enabling keratinocytes to resist physical trauma and to regulate various functions including protection from apoptosis and regulation of immune homeostasis.¹⁸ Hence, cytoplasmic production of CK17 in epidermal keratinocytes adjacent to BCC may have been stimulated by mechanisms related to carcinogenesis as a fundamental stress factor. Much less has been known about the CK19 expression in cutaneous BCC. According to literature^{13,19-21} the immunopositivity has varied between 53.7–88% of cases. In the current paper, the rate of CK19-positive BCCs was lower (40%). In contrast to CK17, the expression of CK19 was only focal and less intensive. A recent study¹⁹ have postulated an interesting theory that cytokeratin profile of BCC may differ according to degree of sun exposure and anatomical localization. It has

demonstrated¹⁹ that CK7 expression was mostly present in non-photoexposed parts of the body. As for CK19 immunostaining, it was found in 73.3% of facial BCCs but only in 43.3% of BCCs arising in non-photoexposed skin.¹⁹ This phenomenon may also depend on the type of skin including a density of cutaneous adnexa. The current study has evaluated too small number of tumors to analyze such a relationship, and all but one CK19-positive BCCs were located on the head. To answer the question whether the expression of certain cytokeratins and another molecular markers in BCC may differ depending on the site of origin might require further science research.

Conclusion

BCC constantly express BerEP4 and CK17 and is variably positive for CK19. In biopsy practice, both BerEP4 and CK17 may serve as reliable markers in the identification and outlining of BCC nests. Since all three markers are strongly positive in the hair follicle but not in the interfollicular epidermis, the staining pattern of CK17, CK19 and BerEP4 in BCC may provide an evidence to the suggestion that the tumor differentiation is toward follicular germinative epithelium.

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