Expression of Two Keratins in Basal Cell, Metatypical, and Squamous Cell Carcinomas of the Skin

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Using an indirect immunoperoxidase method and monoclonal antibodies, the expression of keratins 8 and 17 is investigated in 53 cases of basal cell carcinoma, 10 cases of metatypical carcinoma, and 3 cases of squamous cell carcinoma. Basal cell carcinoma cells are found to express keratin 17 in all cases and keratin 8 in the majority of them. The levels of these keratins in metatypical carcinoma cells are much lower. In squamous cell carcinoma, the keratins are only expressed in a few fields of vision around areas of keratinization. Comparative microspectrophotometric measurements of the intensities of specific staining show that the histological preparations of basal cell carcinoma have optical densities 6-7 times higher than those of metatypical carcinoma and 10-14 times higher than those of squamous cell carcinoma. It is concluded that the features of keratin expression observed in this study may be of use in the differential diagnosis of skin cancers.

Key Words: *skin cancer; keratins; immunohistocytochemistry; anti-keratin antibodies; cytospectrophotometry*

Basal cell carcinoma (BCC) and metatypical carcinoma (MTC) of the skin were recognized as nosological entities relatively recently, although a BCC with morphological characteristics of squamous cell carcinoma (SCC) was described by Darier back in 1922 [4]. In the World Health Organization's International Histological Classification of Tumours, MTC is defined as a tumor in which the type of cells and/or their arrangement cause difficulties in differentiating between BCC and SCC [1]. An MTC may arise either *de novo* or in the presence of BCC, being in the latter case a stage in the progression of a pre-existing tumor [2]. Morphologically, MTC constitute a very heterogeneous group of tumors that differ little from BCC or resemble SCC.

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The features mentioned above complicate the diagnosis of MTC, whose early recognition is crucial because these tumors are prone to metastasize [12]. Efforts are therefore underway to find cell markers capable of reacting selectively with cancers of particular types. One potential marker for cells of epithelial nature is keratin, the major component of intermediate filaments. More than 30 variants of keratin polypeptide chains are known to exist [10], which are variously expressed during the growth and development of keratinocytes [5] and which may therefore be considered for use as markers of tumor cells and to determine the degree of their differentiation. Studies in which BCC and SCC of the skin were examined for the presence of keratins have produced somewhat conflicting results [6,8]. In a relatively recent study, MTC were shown to differ from BCC in the pattern of expression of keratins 8 and 17 [2]. That study, however, was carried out on a limited number of specimens and used cryostatic sections, which create problems in interpret-

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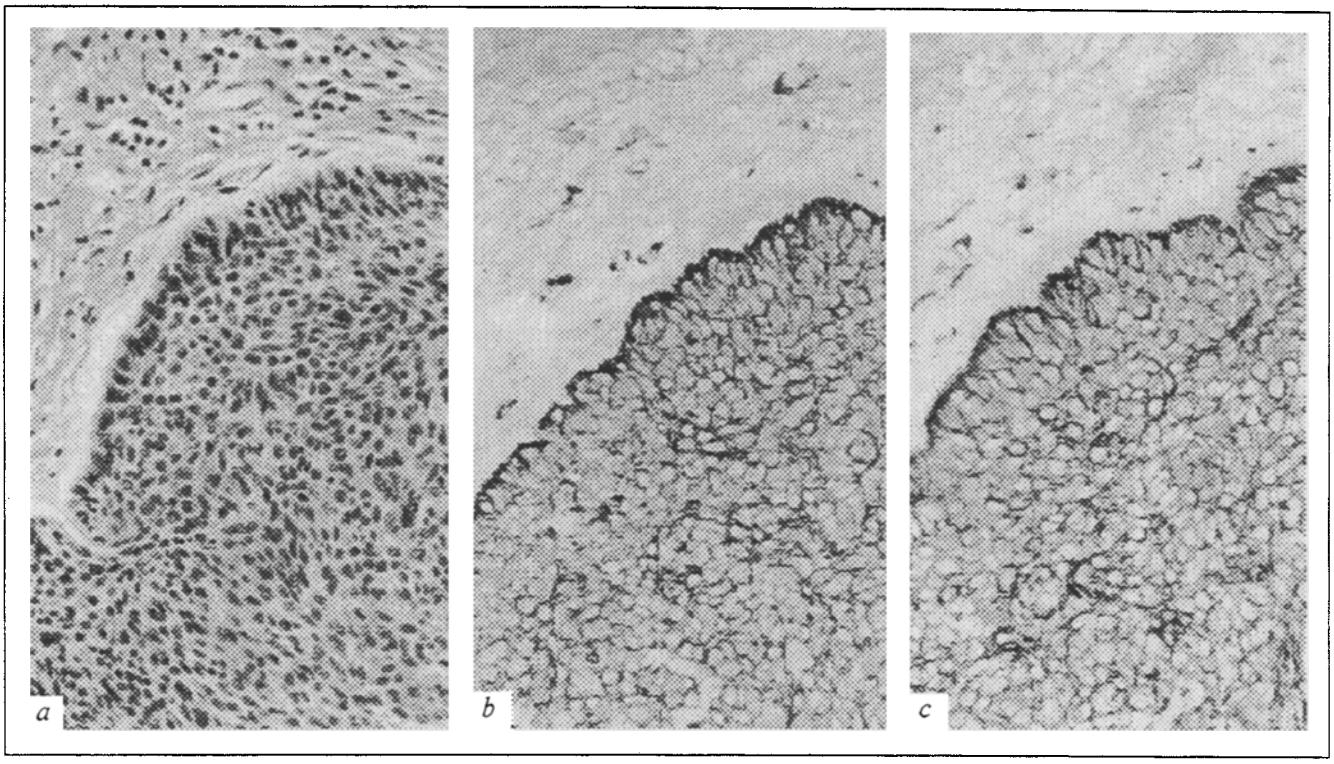

Fig. 1. Basal cell carcinoma of the skin. ×300. Here in Figs. 2 and 3: *a*) hematoxylin–eosin staining; *b* and *c*) demonstration of keratins 17 and 8, respectively, by the immunoperoxidase technique.

ing the morphological picture of the tumor [9] and in identifying areas of metatypy. In the present study, the expression of keratins 8 and 17 in BCC, MTC, and SCC was analyzed immunohistochemically.

MATERIALS AND METHODS

Skin biopsy specimens from 66 patients aged 31 to 75 years were used. BCC had been diagnosed

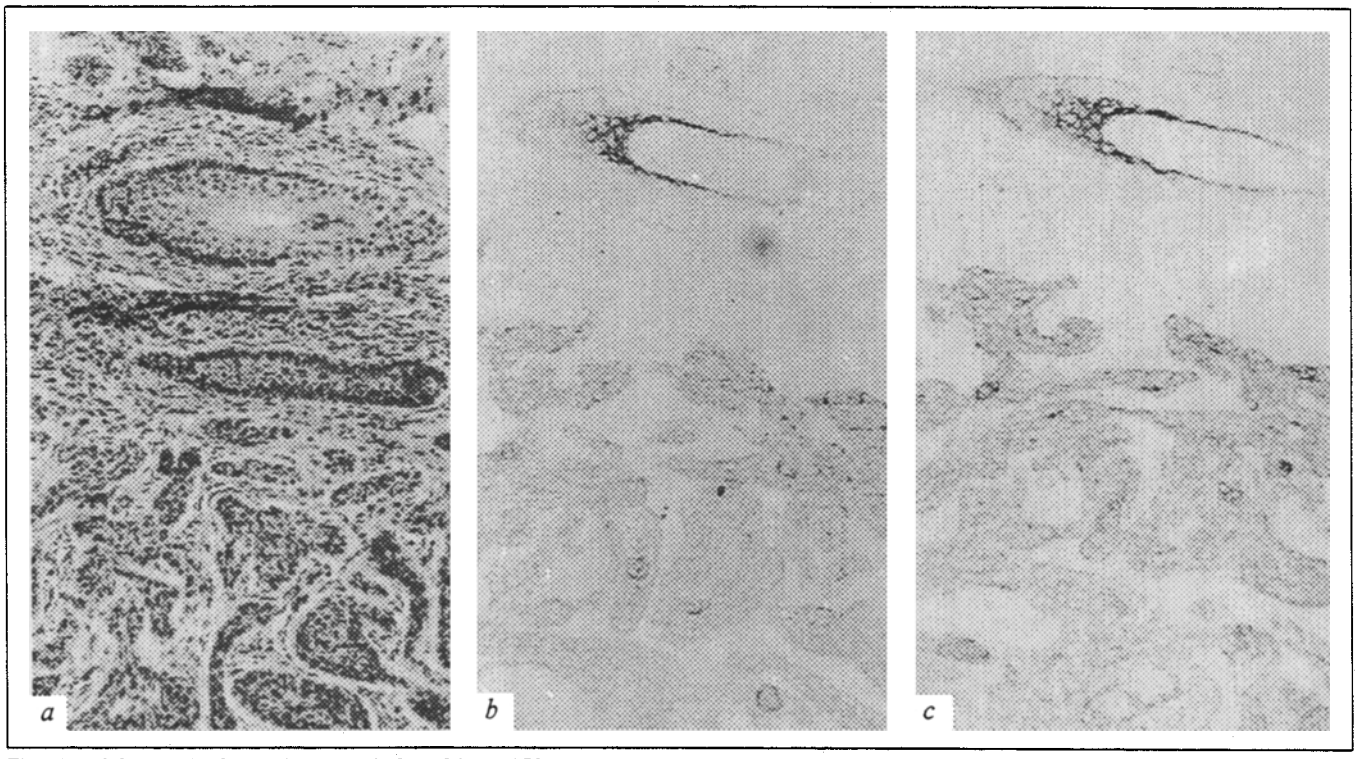

Fig. 2. Metatypical carcinoma of the skin. ×150.

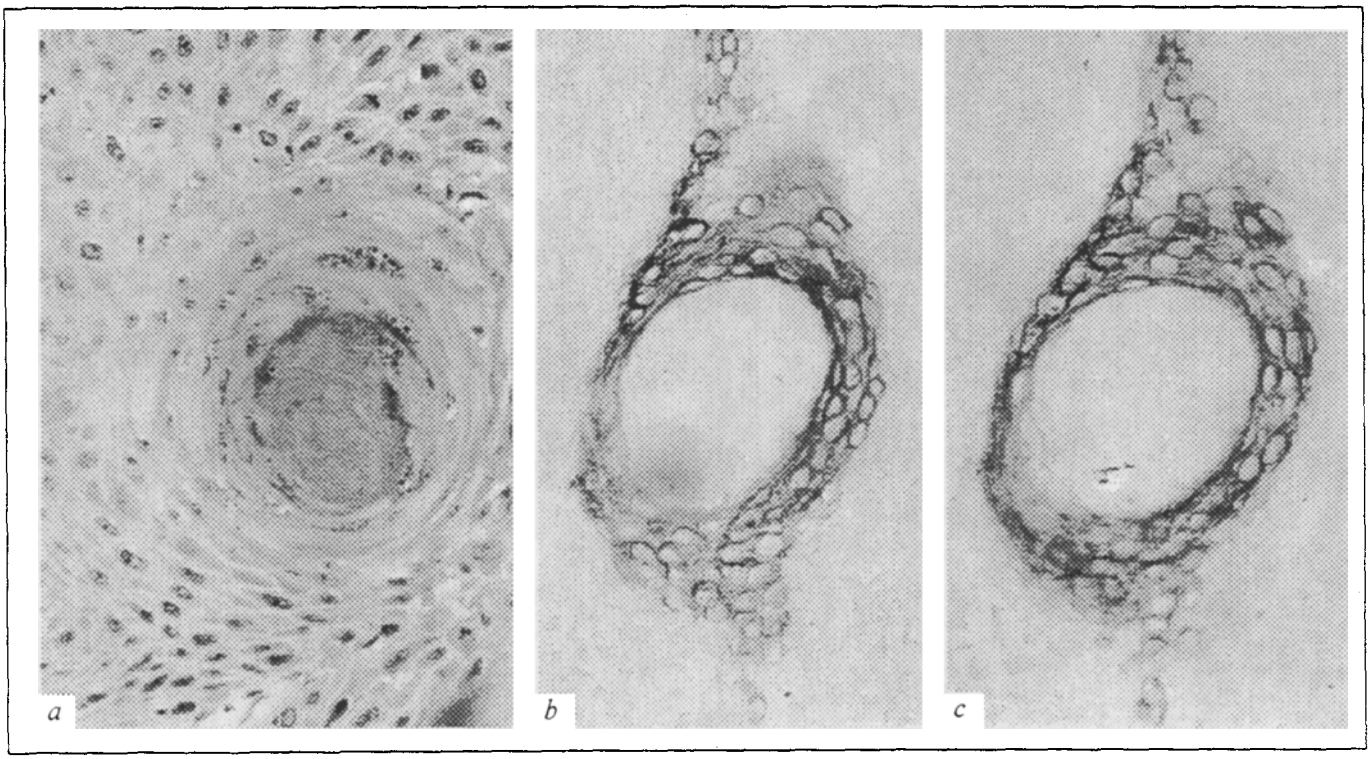

Fig. 3. Squamous cell carcinoma of the skin. ×300.

in 53 cases, MTC in 10, and SCC in 3. Surgical specimens of normal skin were also examined. For the analyses, we used paraffin sections subjected to proteolysis in a 0.1% solution of Pronase E (Merck) for 10 min followed by incubation with a 3% hydrogen peroxide solution for 15 min and a 0.5% bovine serum albumin solution for 30 min. The sections were treated with monoclonal antibodies to keratin 8 and keratin 17 (H1 and E3 clones, respectively [15]) for 30 min, washed, and incubated sequentially with rabbit antibodies to mouse immunoglobulin light and heavy chains and then with sheep antibodies to horseradish peroxidase-conjugated rabbit IgG (Sigma). Peroxidase was detected with 3,3'-diaminobenzidine containing hydrogen peroxide. Control specimens were processed as above except that the IgG fraction from nonimmune mice was used instead of monoclonal antibodies. In nine BCC, MTC, and SCC cases, the intensity of specific staining was measured with an MTsFU-2MP microspectrophotometer (LOMO Company, Russia). For each specimen, 20 sites in the tumor tissue and adjacent areas were analyzed. The results were treated statistically.

RESULTS

In the specimens of normal skin, the antibodies to keratins 8 and 17 stained the sebaceous and sweat glands and also cells in the outer layer of hair follicles. The epidermis failed to react with the antibodies and did not differ from that in control specimens.

In most of the biopsy specimens, preserved epidermis did not generally react with the anti-keratin antibodies, and it was only in some cases that a specific reaction was noted in areas adjacent to cellular complexes of the tumor.

In all BCC specimens, the antibody to keratin 17 produced a specific homogeneous stain forming a thin band along the cytoplasmic periphery. The color was uniformly distributed among all tumor cells to form a reticular pattern sharply differentiated from the surrounding tissue (Fig. 1, *b*). The anti-keratin 8 antibody reacted in a similar way (Fig. 1, *c*) and stained tumors cells well in the large majority of BCC cases; the staining intensity was very weak only in 4 cases.

In all MTC specimens, the antibodies to keratins 8 and 17 gave weak reactions with tumor cells, producing a barely noticeable specific stain which contrasted sharply with the well-stained structures of hair follicles and sebaceous and sweat glands (Fig. 2, *b* and *c*).

SCC cells did not, as a rule, stain with the anti-keratin antibodies used, and it was only in a few fields of vision, mostly around keratinization foci, that specific staining of keratin 17 (Fig. 3, *b*) and, to a lesser extent, keratin 8 (Fig. 3, *c*), was observed.

Comparative cytophotometric measurements of specific staining intensities showed that optical density (absorbance) values for BCC were 6-7 times higher than for MTC and 10-14 times higher than for SCC (Fig. 4).

The results of this study suggest that BCC cells always express keratin 17 and usually also express keratin 8, which distinguishes these cells from the epidermis of normal skin. These results agree with those of other investigators who detected keratin 17 in all BCC [8] and keratin 8 in most [6] or all [2] BCC. The reported absence of keratin 8 expression in skin BCC [7] can be attributed to the use of other antibody clones which probably do not react with altered protein [14]. Further studies will evidently clarify the reasons for these differences.

Keratins 8 and 17 are present in all keratinocytes of the skin during embryogeny [3,5] but subsequently, as the cells differentiate, they disappear from the epidermis while remaining in cells of the hair follicles and of the sebaceous and sweat glands [7,10]. The detection of these proteins in BCC has been interpreted as evidence that this cancer originates from cells of skin appendages [10]. More recent studies have shown, however, that both these keratins are expressed by epidermal cells in *in vitro* culture [13].

The presence of keratin in BCC cells, which is characteristic of tumors in their earlier phases of development, may be a consequence of impaired differentiation of cancer cells.

The much lower (6-7-fold) levels of keratin 8 and 17 expression in MTC as compared to BCC may be an indication that these tumors are less differentiated. In a previous study, neither of these keratins was detected in MTC at all [2], possibly because the specific fluorescence was either very weak or rapidly quenched in the immunofluorescence assay used.

As regards the presence of keratins 8 and 17 in SCC specimens, these proteins were located around keratinization areas, where they were also detected in studies by other authors [7,11], but not in our previous study using immunofluorescence [2]. Apparently, the use of paraffin sections, in which the morphological picture of tissues is preserved, provides a more accurate view of the specific stain distribution.

In MTC, the above-mentioned features of keratin 8 and 17 expression were consistently observed and presented a picture distinct from that seen for BCC and SCC, so that they may be relied upon for differentiating these tumors from other tumors types for diagnostic purposes.

To sum up, BCC cells express keratin 17 in all cases and keratin 8 in most, which distinguishes these cells from epidermal cells of normal skin and from those of MTC, which contain much less (6 to 7 times) keratin. SCC cells express both keratins (8 and 17) just near keratinization foci. The features of keratin expression revealed in this study may be used in the diagnosis of epithelial skin cancers.

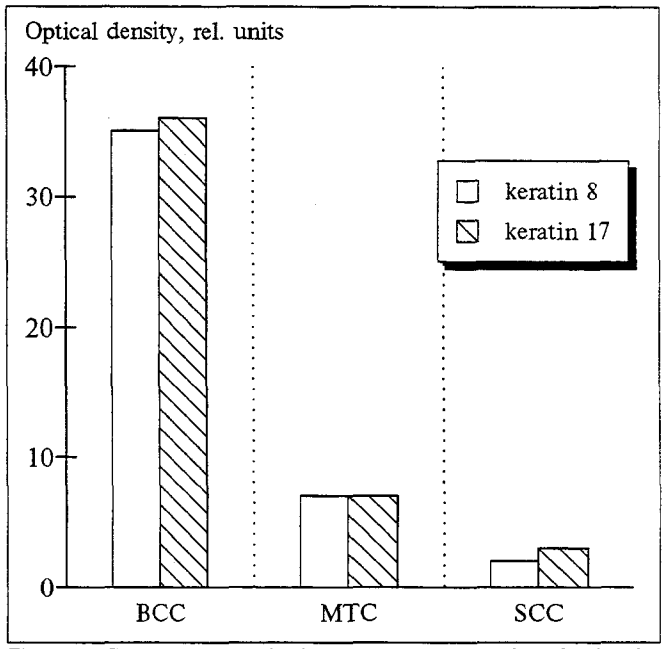

Fig. 4. Comparison of the intensities with which the antibodies to keratin 8 and keratin 17 stained *BCC*, *MTC*, and *SCC* of the skin.

REFERENCES

1. *International Histological Classification of Tumours, № 12: Histological Typing of Skin Tumours*, World Health Organization, Geneva (1980).
2. T. V. Karelina and I. A. Kazantseva, *Arkh. Patol.*, № 11, 29-32 (1991).
3. D. Cooper, A. Schermer, and T. T. Sun, *Lab. Invest.*, **52**, 243-256 (1985).
4. J. Darier, *Brit. J. Dermatol. Syphil.*, **34**, 145-149 (1922).
5. E. B. Lane, J. Bartek, P. E. Purkis, and I. M. Leigh, *Ann. New York Acad. Sci.*, **455**, 241-258 (1985).
6. A. P. Lavrijsen, L. M. Tieben, M. Ponec, *et al.*, *Arch. Dermatol. Res.*, **281**, 83-88 (1989).
7. A. C. Markey, E. B. Lane, L. J. Churchill, *et al.*, *J. Invest. Dermatol.*, **97**, 763-770 (1991).
8. A. C. Markey, E. B. Lane, D. M. Macdonald, and I. M. Leigh, *Brit. J. Dermatol.*, **126**, 154-160 (1992).
9. M. Miettinen, *Pathol. Res. Pract.*, **184**, 431-436 (1989).
10. R. Moll, W. W. Franke, D. L. Schiller, *et al.*, *Cell*, **31**, 11-24 (1982).
11. R. Moll, I. Moll, and W. W. Franke, *Arch. Dermatol. Res.*, **276**, 349-363 (1984).
12. Y. M. Pena, M. M. Bason, and J. M. Grant-Kels, *Arch. Dermatol.*, **126**, 195-198 (1990).
13. H. T. Rupniak, C. Rowlatt, E. B. Lane, *et al.*, *J. Nat. Cancer Inst.*, **75**, 721-735 (1985).
14. H. E. Schaafsma, F. C. S. Ramaekers, *et al.*, *Amer. J. Pathol.*, **136**,, 329-343 (1990).
15. S. M. Troyanovsky, V. I. Guelstein, T. A. Tchipysheva, *et al.*, *J. Cell Sci.*, **93**, 419-426 (1989).