ONCOLOGY

Myoepithelial Differentiation of Basal Cell Carcinoma and Metatypic Skin Cancer

V. R. Babaev, A. N. Khlebnikova, I. A. Kazantseva, and G. Fager

UDC 161.5-006.6-091.8

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 117, № 6, pp. 651-653, June, 1994
Original article submitted September 27, 1993

Basal cell carcinoma and metatypic skin cancer were studied using the indirect immunoperoxidase technique. In basal cell carcinoma morphea-type tumors and in metatypic skin cancer tumor cells contained contractile proteins, specifically, α-actin and smooth muscle myosin.

Key Words: *skin cancer; contractile proteins; myosin; α-actin; antibodies to the nuclear antigen of proliferating cells*

Basal cell carcinoma (BCC), the most common skin tumor, is characterized by a relatively benign course, locally destructive growth, and a low occurrence of metastases [1]. The cases of metastasing BCC so far remain unexplained and they are generally associated with specific variants of cancer [7]. There are detailed reports [11] of some BCC variants, the number of which will obviously increase. Clearly, not only a further search for possible variants of BCC but also a successive analysis of their properties is indicated.

In 1980, WHO experts identified a distinct nosological entity and designated it basal squamous or metatypic skin cancer (MSC). This cancer differs from BCC in a greater degree of malignancy, which is manifested in a considerable increase of the mitotic index and the number of pathological mitoses [2]. The objective difficulties of diagnosing MSC on histological preparations stained by routine methods motivate the search for additional specific cell markers for the differentiation of MSC, BCC, and their variants. It is possible that specific features in the expression of some cytoskeletal proteins can serve as such markers.

In the present study we analyzed the expression of α-actin, myosin, desmin, and vimentin in BCC and MSC. We also studied the proliferative activity of cancer cells by a histoimmunochemical method with the use of antibodies to the nuclear antigen of proliferating cells.

MATERIALS AND METHODS

Skin bioptates obtained from 50 patients aged 31-80 years were used in the study. The bioptates were histologically classified by the appropriate criteria [5]. There were 42 cases of BCC (solid type 25, morphea type 7, solid adenoid 5, and adenoid 1) and 8 cases of MSC. Serial paraffin sections were deparaffinated, and treated with 3% H_2O_2 and 0.5% bovine serum albumin for 20 min with each solution. They were then incubated (24 h at 4°C) with monoclonal antibodies to the smooth muscle α-actin, including IA4 (Sigma) and HHF35 [10] clones, to desmin, vimentin (both Calbiochem),

Institute of Human Morphology, Russian Academy of Medical Sciences; Moscow Region Research Clinical Institute, Moscow; Wallenberg Laboratory of Getheborg University, Getheborg. (Presented by N. K. Permyakov, Member of the Russian Academy of Medical Sciences)

0007-4888/94/0006-0656$12.50

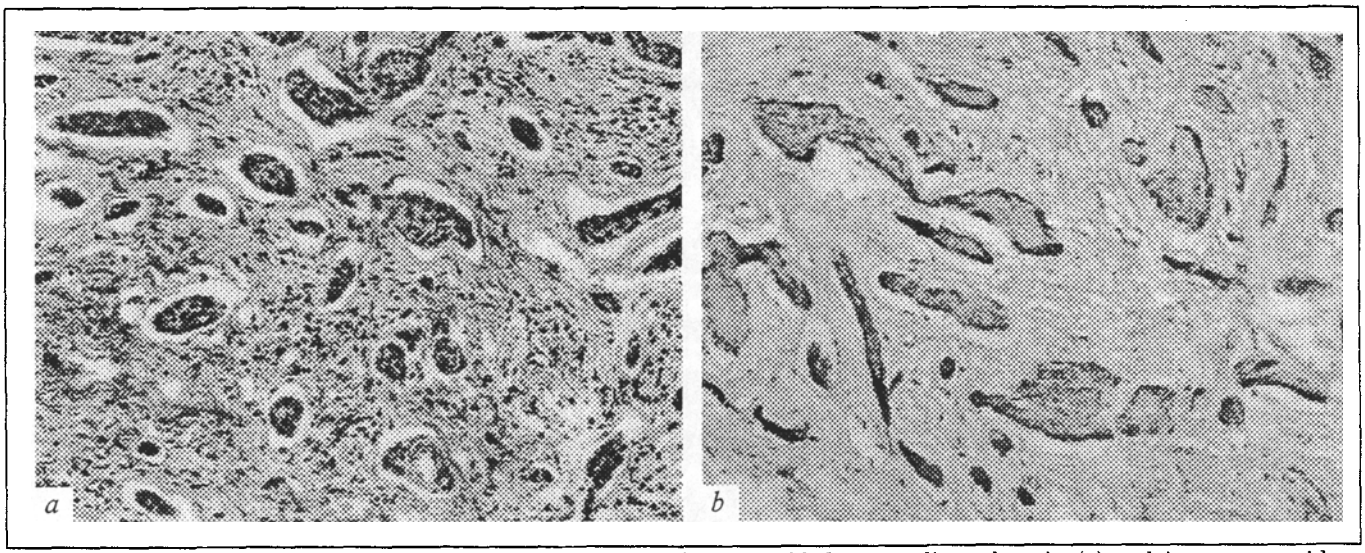

Fig. 1. Morphea-type BCC containing contractile proteins. Staining with hematoxylin and eosin (*a*) and immunoperoxidase identification of α-actin (*b*). ×125

and to the nuclear antigen of proliferating cells (PC 10, Dako) [12]; some of the sections were incubated with rabbit antibodies to smooth-muscle myosin [4]. The sections were washed and successively treated with rabbit antibodies to the light and heavy chains of mouse immunoglobulins and sheep antibodies to rabbit IgG conjugated with horseradish peroxidase (Sigma) diluted 1:100 in normal saline. The sections were developed with 3,3'-diaminobenzidine containing H_2O_2. Control preparations were treated according to this scheme, but monoclonal antibodies were replaced with nonimmune mouse IgG. Counting was performed at a 100-fold magnification on preparations stained with hematoxylin. For BCC and MSC the total number of nuclei and nuclei labeled with peroxidase were counted in 10 fields of view.

RESULTS

In 8 cases of a basalioma (16.6%) and 3 cases of MSC (37.5%) tumor cells reacted with anti-α-actin monoclonal antibody in histological preparations of skin bioptates. The morphea-type BCC displayed the highest (4 of 7 cases) occurrence of α-actin (Fig. 1); this was lower in solid-adenoid (1 of 5 cases), and was lowest in solid BCC (3 of 25 cases). The reaction was manifested in a homogeneous staining distributed all through the cytoplasm with the exception of the nuclear zone (Fig. 2). In most cases (5 BCC and 2 MSC) α-actin was detected in practically all tumor cells, which varied in the intensity of specific staining. In two basaliomas α-actin was detected only in some cells, which were located randomly. In addition, in one basalioma and in one MSC the specific α-actin staining was seen in the cells located at the periphery on the tumor nodes.

Subsequent staining of the sections with the other clone of anti-α-actin antibody (HHF 35) and with anti-myosin antibodies showed that these antibodies react with the cells of the same cancer type and stain them in a similar manner. Anti-vimentin antibodies stained all the tumor cells, whereas anti-desmin antibodies did not react with tumor cells and stained only small blood vessels in the derma.

In all cases of BCC and MSC the antibodies to the nuclear antigen of proliferating cells stained some (6.5-42%) of the tumor cell nuclei. This specific staining varied in intensity and was distributed as granules in nucleoplasm. Generally, the

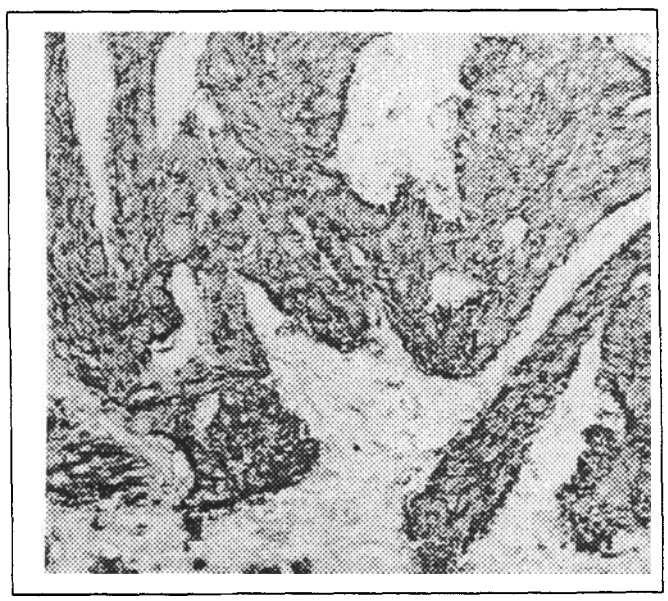

Fig. 2. Distribution of α-actin in BCC cell complexes (immunoperoxidase staining, ×320).

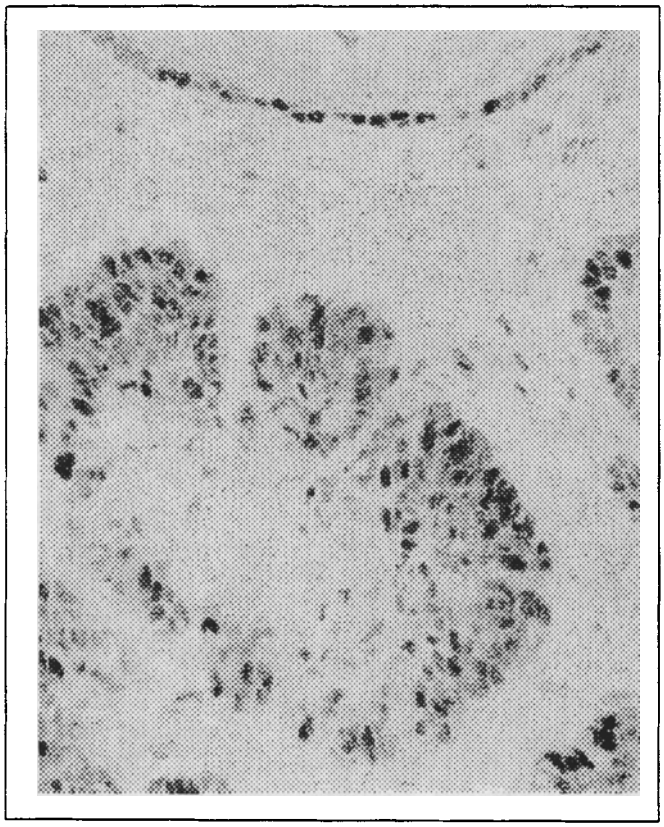

Fig. 3. Distribution of proliferating cells in MSC cell complexes. Immunoperoxidase staining with antibodies to nuclear antigen of proliferating cells. ×320.

proliferating cells were evenly distributed over the entire tumor; however, in one basalioma and in one case of MSC they were localized at the periphery of the tumor nodes, which, incidentally, coincided with a similar distribution of the cells containing α-actin (Fig. 3).

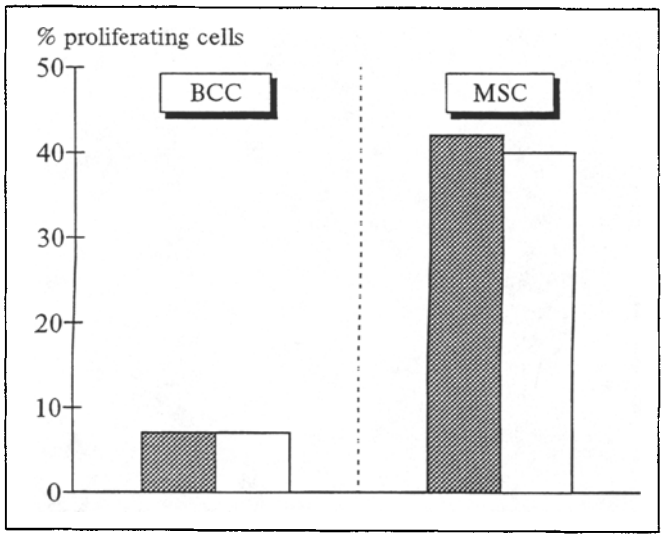

Fig. 4. Comparative analysis of proliferative activity of BCC and MSC cells, with (black bars) and without (white bars) contractile proteins.

A quantitative comparison of the number of proliferating cells in the tumors with and without contractile proteins revealed no significant differences between them (Fig. 4).

The results obtained indicate that in some cases of BCC and MSC the tumor cells contained contractile proteins, specifically α-actin and smooth-muscle myosin. These proteins were detected in the large majority of tumor cells, which excludes the possibility of erroneous identification. Myofilaments in morphea-type BCC were revealed earlier by electron microscopy [6]. Similar actin-containing cells have been recently detected by immunochemical methods in 5 cases of BCC and were claimed to be a consequence of myoepithelial differentiation of tumor cells [9]. The possibility in principle of myoid differentiation of epithelial tumors of other localization has been demonstrated in a number of studies [8].

Current concepts of carcinogenesis presuppose a genetic basis of the pathology with a vast array of causes and mechanisms of tumor transformation [15]. Multiple genetic alterations may occur either in crucial regulatory genes or in the genes suppressing tumor growth [14] or responsible for DNA repair [13]. This suggests the existence of numerous variants of BCC, as was postulated earlier [11] and then proved by a number of investigations [3,9]. Consequently, myoepithelial differentiation can be regarded as one of the numerous variants of BCC. It is quite possible that a similar phenotype of keratinocytes exists in health, and genetic transformation just stabilizes this kind of transformation.

The use of antibodies to the nuclear antigen of proliferating cells allowed us to compare tumors with and without contractile proteins. These tumors displayed virtually the same proliferative activity. However, the proliferating activity of MSC was considerably higher than that of BCC, which confirms the results of previous studies [2].

Thus, in some cases of BCC and MSC the tumor cells contained contractile proteins (α-actin and myosin), which may be a consequence of their epithelial differentiation.

The proliferative activity of the tumors whose cells contained contractile proteins did not differ from that of BCC and MSC which did not express these proteins.

REFERENCES

1. A. K. Apatenko, *Epithelial Tumors and Abnormalities of Skin Development* [in Russian], Moscow (1973).
2. I. A. Kazantseva, I. I. Bogatyreva, A. M. Vavilov, and S. M. Parshikova, *Arkh. Patol.*, №6, 35-39 (1983).

3. N. T. Raikhlin and B. M. Korkmazov, *Ibid.*, № 9, 29-35 (1985).
4. V. R. Babaev, A. S. Antonov, O. S. Zakharova, *et al.*, *Atherosclerosis*, **71**, 45-56 (1988).
5. W. F. Lever and G. Schamburg-Lever, *Histopathology of the Skin*, 6th ed., Lipincott, Philadelphia (1983).
6. N. S. McNutt, *Lab. Invest.*, **44**, 359-367 (1981).
7. S. J. Miller, *J. Amer. Acad. Dermatol.*, **24**, 1-13 (1991).
8. J. J. Sciubba and R. B. Brannon, *Cancer*, **49**, 562-572 (1982).
9. S. Suster and C. S. Ramon, *Amer. J. Dermatopathol.*, **13**, 350-357 (1991).
10. T. Tsukada, D. Tippens, D. Gordon., *et al.*, *Amer. J. Pathol.*, **126**, 51-60 (1987).
11. T. R. Wade and A. B. Ackerman, *J. Dermatol. Surg. Oncol.*, **4**, 23-28 (1978).
12. N. H. Waseem and D. P. Lane, *J. Cell Sci.*, **96**, 121-129 (1990).
13. Q. Wei *et al.*, *Proc. Nat. Acad. Sci. USA*, **90**, 1614-1618 (1993).
14. R. A. Weinberg, *Science*, **254**, 1138-1146 (1991).
15. C. L. Willman and C. M. Fenoglio-Preiser, *Human Pathol.*, **18**, 895-902 (1987).

The Uptake of 3-Deoxy-3-Iodine-Glucose by Normal and Tumor cells *In Vitro*

V. P. Kharchenko, V. K. Bozhenko, E. V. Khmelevskii, A. M. Shishkin, and A. G. Zakharov

UDC 616-006.04-092-07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 117, № 6, pp. 654-655, June, 1994
Original article submitted November 26, 1993

It is found that 3-deoxy-3-iodine-glucose (3D-I-glucose) enters both erythrocytes and tumor cells at a rate close to that of glucose entry. For tumor cells the total uptake of the preparation was 6-fold higher than for erythrocytes (1500 and 250 μM/mln. cells, respectively). Phosphorylated products of 3D-I-glucose were not detected; however, the total amount of preparation in the probes dropped during incubation, indicating that it is metabolized by the cells.

Key Words: *3D-I-oxyglucose; human erythrocytes; tumor cells*

The metabolism of saccharide derivatives is important and interesting from the viewpoint of applied problems of diagnostics and treatment of malignant neoplasms [1,3,4]. At the present time modified saccharides, for example, 2-fluorine-D-glucose, have found application not only in experimental oncology but also in clinical practice [5,6]. At the same time, the metabolic peculiarities of the majority of saccharides have so far not been studied. It should be noted that, theoretically, iodine derivatives of glucose may carry both a diagnostic marker and therapeutic activity (^{125}I and ^{131}I) to tumor tissues.

Institute of Diagnostics and Surgery, Ministry of Public Health; State Institute of Physico-Technical Problems, Russian Academy of Sciences, Moscow

In the present work we studied the uptake of 3-deoxy-3-iodine-glucose by normal and tumor cells *in vitro*.

MATERIALS AND METHODS

3D-I-glucose was synthesized at the Zelinskii Institute of Organic Chemistry, Russian Academy of Sciences. Its stability was checked by NMR spectroscopy. The concentration of 3D-I-glucose was measured by the orthotoluidine method [2]; the sensitivity of this method to the preparation is somewhat higher than to glucose. Since the method is sensitive to all reduced saccharides, the contribution of glucose was determined in an Eksan-G analyzer (PZTM, Lithuania) based on the principle of a glucose oxidase electrode (3D-I-glucose cannot be

0007-4888/94/0006-0659$12.50