ONCOLOGY

Expression of Peripheral Benzodiazepine Receptor, PCNA, and Caspase-3 in Cells of Skin Melanoma and Squamous Cell Carcinoma

T. G. Ruksha, A. B. Salmina, V. D. Sokolov, T. V. Maksimova, and Yu. A. Anisimov

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The intensity of cell proliferation and apoptosis and expression peripheral benzodiazepine receptor were studied in skin biopsy specimens from patients with squamous cell carcinoma and melanoma of the skin. Sharp inhibition of apoptosis and changes in the levels of cell proliferation in tumor cells were paralleled by decreased expression of peripheral benzodiazepine receptor.

Key Words: *peripheral benzodiazepine receptor; apoptosis; cell proliferation; melanoma of the skin; squamous cell carcinoma of the skin*

A pronounced increase in the incidence of malignant tumors of the skin [1] necessitates further studies of the pathogenesis of this group of diseases in order to improve their diagnosis and therapy. Peripheral benzodiazepine receptor (PBR) is an intracellular protein with a molecular weight of 18 kDa located on the outer mitochondrial membrane. PBR is involved in the transport of cholesterol and some proteins through the mitochondrial membrane and regulates cell proliferation, apoptosis, and synthesis of steroid hormones [4]. Recent studies showed that UV can modify the structure and properties of PBR [3]. Hence, PBR in skin cells can participate in modulation of cell proliferation and apoptosis induced by UV light and therefore can be a potential therapeutic target in malignant tumors of the skin.

We evaluated the expression of PBR, PCNA, and caspase-3 in normal and malignant skin cells.

MATERIALS AND METHODS

Skin specimens from healthy volunteers (n=6) and patients with squamous cell carcinoma of the skin (SCS; n=14) and malignant skin melanoma (MSM; n=12) were fixed in 10% neutral formalin. Histological sections (up to 5 μ) were stained for immunohistochemical study by the standard protocol [2]. Monoclonal antibodies to PBR [7] (a kind gift from Prof. V. Papadopoulos, Georgetown University, Washington), PCNA (proliferating cell nuclear antigen), and caspase-3 (Novocastra Laboratories Ltd.) were used. Before incubation with antibodies to PCNA and caspase-3, the antigen was demasked by incubation in citrate buffer at 89°C (Retrievagen, BD Biosciences). Second antibodies labeled with horseradish peroxidase (Ready-to-Use System, Novocastra Laboratories Ltd.)

Department of Biochemistry with a Course of Medical Chemistry, Krasnoyarsk State Medical Academy, Federal Agency for Public Health and Social Development; Department of Clinical Morphology, Krasnoyarsk Territorial Bureau of Pathology. ***Address for correspondence:*** tatyana_ruksha@mail.ru. T. G. Ruksha

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and diaminobenzidine (BD Biosciences) as a chromogene were used for detection of the antigen, after which the specimens were poststained with hematoxylin. The numbers of positively stained cells per 100 tumor cells (in studies of the epidermis per 100 epidermal cells) were counted.

The results were statistically processed using Mann—Whitney nonparametric test.

RESULTS

In normal epidermis, PBR was distributed homogeneously and perinuclearly. PBR were detected in cells of sebaceous and sweat glands and in the papillary layer of the derma. The intensity of expression was 92 per 100 epidermal cells. The expression of PBR in the epidermis in SCS patients was significantly lower than in the control (Table 1), especially in its basal layer. In tumor foci PBR was expressed evenly. No appreciable differences between the level of PBR expression in normal skin cells and SCS cells were detected, but staining intensity in PBR^+ tumor cell was significantly below the control. In patients with MSM, the level of PBR expression in the basal layer of intact epidermis decreased. The expression of PBR in tumor cells was lower than in the epidermis and significantly lower than in the control group (Table 1).

Since PBR are involved in the regulation of cell proliferation, we measured the level of PCNA expression correlating with the intensity of cell growth and proliferation in the skin [8]. In normal skin epidermis, PCNA was detected in the nuclei of basal layer cell. In biopsy specimens from patients with malignant tumors of the skin, PCNA was expressed in tumor tissue and in intact epidermis (Table 1). The expression of PCNA was much more intensive in biopsy specimens from patients with SCS. The antigen was detected in the intact skin epidermis (in basal and prickle-cell layers). Expression of PCNA in tumor complexes was homogeneous and evenly intensive in the entire tumor focus. In MSM, PCNA was detected in the epidermis mainly in the basal layer cells. In tumor foci, PCNA was detected in nuclei of atypical cell, but the intensity of its expression greatly varied and no appreciable differences between PCNA levels in normal skin and MSM cells were detected (Table 1).

Changes in cell proliferation are often paralleled by changes in apoptosis, because these two opposite physiological phenomena are interrelated. The intensity of apoptosis was assessed by the level of caspase-3 (effector caspase, whose activity increases during the development of irreversible events in apoptosis). In epidermis of normal skin, caspase-3 was detected in the basal layer, in cell cytoplasm. In SCS, caspase-3 expression in the epidermis sharply decreased, the intensity of positive staining was significantly lower in normal skin. The enzyme was virtually not detected in tumor tissue: just solitary slightly stained cells were detected. Solitary slightly positive cells with expression of caspase-3 were detected in the basal layer of the epidermis in MSM in tumor cells (Table 1).

Hence, cell proliferation/apoptosis ratio is changed in malignant tumors of the skin, which is characteristic of many tumor diseases: the pool of proliferating cells increases and the intensity of apoptosis decreases. The changes of apoptosis in SCS and MSM are similar. However, MSM is a more aggressive tumor than SCS. Presumably, this is due to different sensitivity of these tumors to apoptosis-inducing stimuli.

The expression of PBR was low in SCS and MSM cells. The data indicate changes in PBR functioning in skin cells during their malignant transformation. We cannot explain the decrease in PBR expression, because this parameter increased in the majority of malignant tumors [5]. For example, increased expression of PBR correlates with high proliferative potential and enhanced cholesterol transport in mammary carcinoma cells [6]. In pso-

TABLE 1. Expression of PBR, PCNA, and Caspase-3 in the Skin of Healthy Individuals and Patients with Malignant Tumors of the Skin

Object of study		PBR^+ cells, %	$PCNA^+$ cells, %	Caspase-3^+ cells, %
Control		92.0	9.0	19.0
SCS	normal epidermis	57.0*	31.4	1.3*
	tumor tissue	83.0	64.5*	0*
MSM	normal epidermis	28.7*	28.7	6.0*
	tumor tissue	19.8*	31.2	0.95*

Note. *$p<0.01$ compared to the control.

riasis, cell proliferation is also high and the level of PBR expression positively correlates with mitotic activity of epidermal keratinocytes [2]. Presumably, tumor transformation of skin cells is associated with disorders in the regulation of PBR functioning and expression. Further studies will clear out the role of PBR in the skin in health and disease. The SCS and MSM cells can be adequate models for studies of PBR functioning during the development of malignant tumors and skin changes induced by UV light.

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