CELLULAR LOCALIZATION OF HUMAN SECRETORY β-GLOBULIN IN NORMAL AND TUMOR TISSUES

Yu. S. Tatarinov, A. K. Tagirova, K. K. Pugachev, I. B. Shimbireva, and A. A Terent'ev

UDC 616-006-018-008.939.624-078.33

KEY WORDS: steroid-binding proteins; immunohistochemistry; tumors.

The writers previously described a steroid-binding secretory β-globulin (SBG), found in high concentration in seminal plasma [2]. Besides seminal fluid, this antigen also has been detected In saliva and in very small amounts in human milk. A change in the level of specific transport proteins in biological fluids is known to lead to disturbance of hormonal homeostasis and to be a risk factor of tumor development [4, 5]; for that reason, determination of the cellular localization of SBG in normal and malignant tissues could shed light on the role of this antigen in the mechanism of hormonal homeostasis.

The aim of this investigation was an immunohistochemical study of the distribution of SBG in definitive and embryonic tissues, and also in some human tumors.

EXPERIMENTAL METHOD

SBG was isolated from human seminal plasma by affinity chromatography on estradiol-triazine-sepharose, on the basis of affinity for immobilized estradiol [2] Antibodies to SBG were obtained from rabbit antiserum on an immunosorbent, which was synthesized by adding SBG to sepharose CL-4B through 4β-hydroxyethylsulfonyl-2-aminoaniline sulfate ester [1]. Antibodies were eluted by 0.15 M NaCl, acidified with HCl to pH 2.55.

The test antigen was determined by an indirect histochemical method of enzyme immunoassay [3]. The histological material was prepared by fixation in 10% buffered formalin followed by embedding in paraffin wax. The sections were 5-6 μm thick. Before immunochemical processing the sections were dewaxed, after which they were treated with first-order rabbit antibodies in a concentration of 5 mg/liter A conjugate of donkey antibodies against rabbit γ-globulins with peroxidase, used in a dilution of 1:80, served as secondary antibodies. Nonspecific binding was suppressed by preliminary treatment of the sections with horse serum. Endogenous peroxidase actively was blocked by 3% H_2O_2. The action of peroxidase was visualized with the aid of 3'3'-diaminobenzidine tetrachloride. The sections were counterstained with hematoxylin. The specificity of the reaction was verified by treating the sections with nonimmune serum, substrate, and second-order antibodies. Sections through the seminal vesicles served as the positive control.

EXPERIMENTAL RESULTS

The study of normal tissues revealed SBG only in adults (Table 1). The reaction was most intensive in the epithelial cells of the seminal vesicles. SBG was localized in the apical zone of the cytoplasm of the high cylindrical epithelium

Department of Biochemistry, N. I. Pirogov Second Moscow Medical Institute P. A. Gertsen Moscow Oncologic Research Institute (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. S. Lopukhin.) Translated from Byulleten Éksperimental'noi Biologii i Meditsiny, Vol. 113, No 1, pp. 79-82, January, 1992. Original article submitted February 1, 1991.

TABLE 1. Immunohistochemical Determination of SBG in Normal Fetal and Definitive Tissues

Tissues	Number of observations	Number of positive cases
1. Definitive:		
a) mammary gland	8	2
b) seminal vesicles	2	2
c) salivary glands	3	3
d) salivary glands	2	2
e) prostate	1	1
f) other: liver, brain msucles, adrenal, thyroid gland, testis, uterus urinary bladder, large and small intestine, stomach, pancreas, esophagus, thumus, spleen, skin	38	0
2. Fetal and embryonic: lung, liver, brain, adrenal, thyroid gland, uterus, urinary bladder, large and small intestine, stomach, pancreas, esophagus, spleen, skin	51	0

TABLE 2. Distribution of SBG in Tumor Tissues

Localization of tumor	Number of positive cases	Number of positive cases
1. Mammary gland		
Carcinoma	32	5
Epithelium of ducts outside focus of carcinoma	14	14
Tissue outside focus of carcinoman, including epithelium of lobubles	14	0
Mastopoathy	1	0
2. Lung		
Squamous-cell carcinoma	3	0
Small-cell carcinoma	6	0
Adenocarcinoma	12	0
Bronchia gland soutsie focus of carcinoma	10	1
3 Salivary gland		
Pleomorphic adenoma		
4. Other tissues	1	1
Carcinoma of stomach, large intestine, body and cervix of uterus, ovaries. kidney, larynx sarcoma ma, leiomyoma, melanoma		

in the form of small granules. The antigen was found also in the lumen of the ducts. In the serous salivary glands a positive immunoperoxidase reaction was localized in the supranuclear region of the epithelial cells and in the secretion (Fig. 1). Investigation of prostatic tissue revealed a weaker reaction in the glandular epithelial cells and in the lumen of the small ducts. Expression of SBG in the epithelial cells of the mammary gland was of average intensity and was discovered in only two of the specimens studied. SBG was found in the lining of the small and medium-sized ducts in the apical part of the cytoplasm in the form of intracellular granules. In the acini no immunoperoxidase reaction was observed. In the serous glandular cells of the bronchial epithelium staining was weak, but distinct. In the remaining tissues of adults studied no antigen was found.

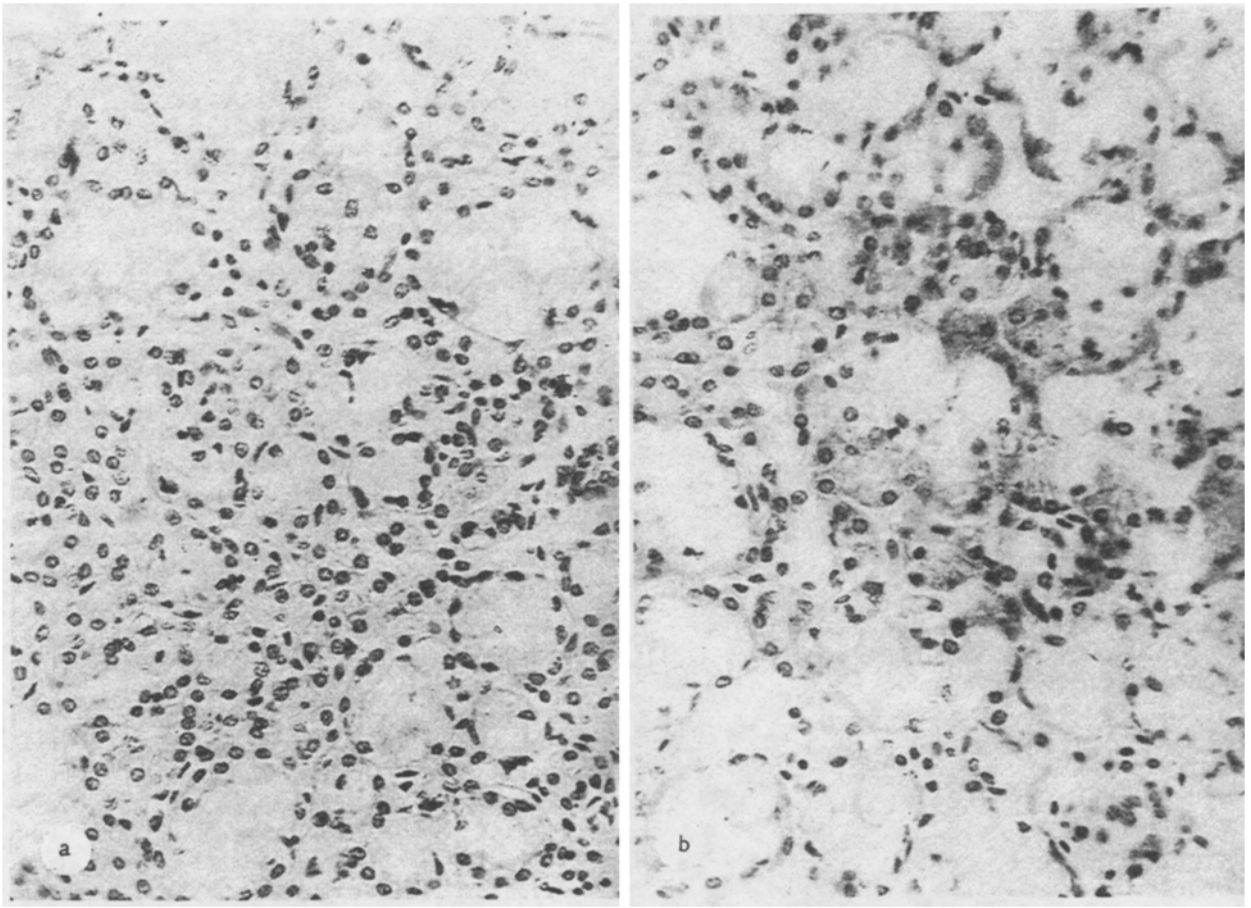

Fig. 1. Immunohistochemical demonstration of SBG in cells of serous epithelium of salivary gland. a) Unstained control section, incubated with nonimmume serum; b) experiment. Strong immunoperoxidase reaction localized in cytoplasm of epithelial cells of salivary gland. Magnification 300× Counterstained with hematoxylin.

In the tissues of human fetuses aged 9-12 weeks and in the later fetal period until 32 weeks no SBG was found (Table 1). This suggests that SBG is a definitive antigen and is synthesized in differentiated, mainly hormone-dependent, tissues.

The study of expression of SBG in cells normally containing this protein and which have undergone malignant change is of definite interest, especially in the case of the most widespread forms of breast and lung cancer. During a study of lung cancer, expression of SBG was not found in one of 21 cases, and the reaction was absent, moreover, not only in the cancer cells, but also in cells of the bronchial glands, which normally give a positive reaction for SBG. Only in one case close to a focus of carcinoma was very weak staining found in the bronchial glands outside the focus of malignant transformation (Table 2). Absence of expression of SBG in lung cancer points to loss of this protein by the malignant cells. Loss of the reaction in bronchial tissue surrounding a focus of neoplasia may perhaps be connected with the effect of malignantly changed cells on nearby surrounding tissues.

This antigen also was discovered in a pleomorphic salivary adenoma in the apical part of the cytoplasm and the contents of the ducts.

In a study of malignant mammary gland tumors a significant ($p < 0.001$) increase was found in expression of SBG in the epithelium of the ducts outside a focus of malignant change. A positive reaction was found in cystically dilated ducts close to malignant foci, and in some cases seepage of secretory antigen was observed into the connective tissue surrounding the ducts. Any positive immunoperoxidase reaction also was localized in cells of a carcinoma invading the ducts, whereas in lobular carcinoma this antigen was absent. The antigen was localized in neoplastic structures as a rule in the apical zone, but in some cases it filled the whole cytoplasm of the cells (Fig. 2).

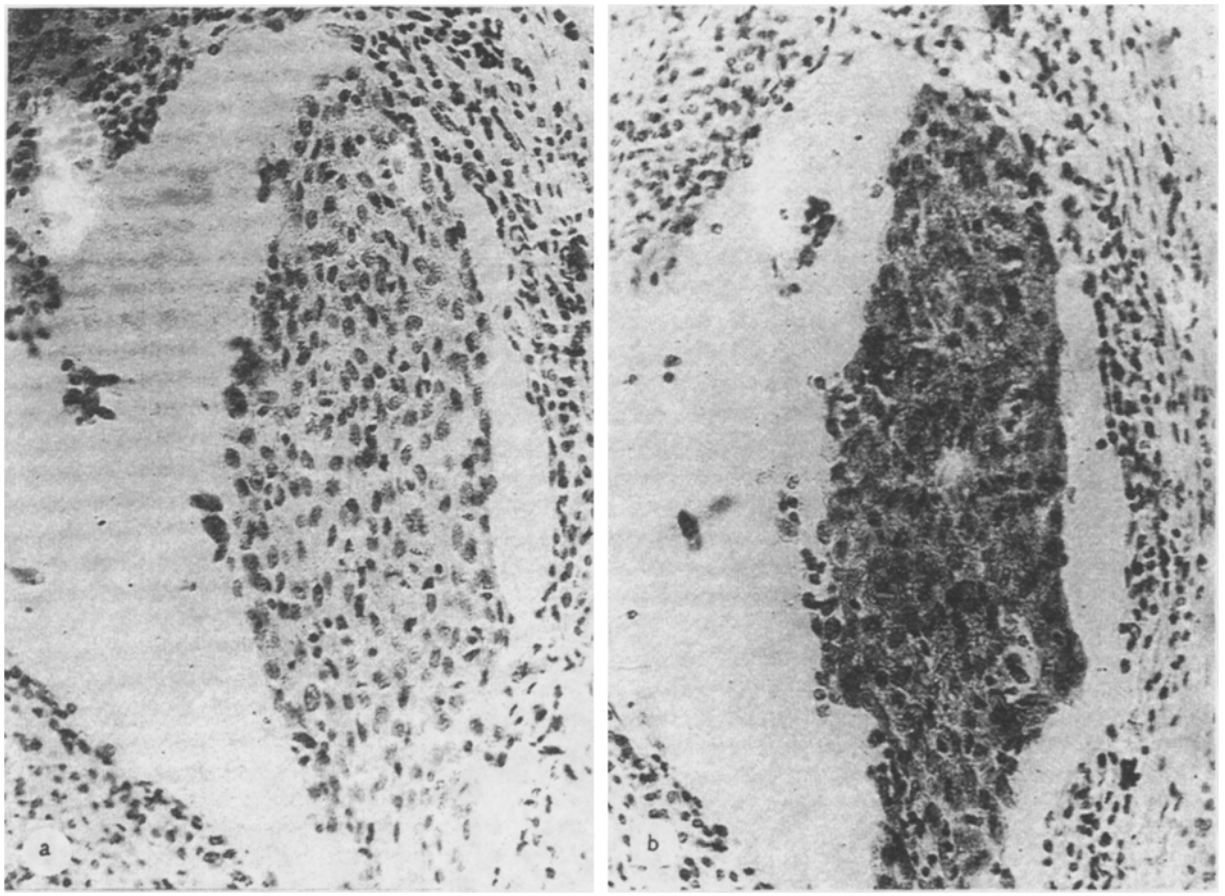

Fig. 2. Staining of SBG-positive cells in tissue of a mammary gland carcinoma a) negative control section, incubated with nonimmune serum; b) experiment. Positive diffuse reaction within cytoplasm of tumor cells. Magnification 300×, counterstained with hematoxylin.

Receptors for estrogens are preserved in 50% of mammary gland carcinoma cells [5]. It is in such cases that removal of estrogens often leads to remission. The possibility cannot be ruled out that the hormonal dependence of malignant mammary gland cells is connected with the presence of SBG in them, and also with a significant increase in the expression of this protein close to a focus of carcinoma. Production of SBG, possessing affinity for sex steroids may prove to be an essential step in processes of recognition and reception in hormone-sensitive cells.

By contrast with mammary gland carcinoma, morphological and functional disturbances in lung tumors during malignant change lead to loss of SBG not only in carcinoma cells, but also in surrounding tissues. Characteristics of this kind may serve as an example of negative marking of neoplastic transformation.

It was thus demonstrated immunohistochemically that SBG possesses marked tissue specificity and is produced in secretory epithelial cells. The highest SBG level is found in the seminal vesicles and salivary glands, and it is synthesized less intensively in tissues of the mammary gland and prostate. A weak immunoperoxidase reaction is found in the epithelium of the bronchi. In fetal and embryonic tissues SBG is not produced. It can be concluded that SBG is a definitive marker of secretory cells in the late stages of differentiation In neoplasms SBG synthesis is disturbed: it remains partially expressed in cells of mammary gland carcinoma and it is expressed intensively in the epithelium of the ducts of the mammary gland close to a focus of malignant transformation. In cells of lung cancer and in bronchial glands close to a focus of neoplastic transformation, the protein is absent.

LITERATURE CITED

1. A. E Gurvich and O. B. Kuzovleva, Immunochemical Analysis [in Russian], ed. by L. A. Zil'ber, Moscow—Leningrad (1968), pp. 54-67.
2. Yu. S. Tatarinov, A. K. Tagirova, and A. A. Terent'ev, Byull. Éksp. Biol. Med., No. 11, 469 (1990).

3. K. Heyderman, J. Clin. Pathol., **32**, No. 1, 971 (1979).
4. J. P. Janssens, Cancer, **55**, No. 1, 2600 (1985).
5. J. Moore, T. J. A. Key, G. M. G. Clark, et al., Steroids, **52**, No. 4, 391 (1988).

ERYTHROPHAGOCYTOSIS AND PIGMENTED CELLS OF THE AMPHIBIAN LIVER

M. M. Kalashnikova

UDC 612.35-576.3-591.111.1-591.111.7-597.61.9

KEY WORDS: erythrophagocytosis; pigmented liver cells.

It is now established that pigment granules are not only accumulated, but are also synthesized in Kupffer cells [4, 5, 9]. Pigment granules have been shown to contain melanin, hemosiderin, and lipofuscin [2, 5], and the protein matrix of pigment granules possesses tyrosinase activity [10, 5], which is higher in winter and lower in summer. The melanin content reaches a maximum in the cold months of the year, and a minimum in the hot period [10, 5]. The question of the role of the pigmented cells of the liver and the causes of seasonal differences in melanin content remains unanswered. By studying the ultrastructure of the liver cells of adult frogs during hibernation and of tadpoles during metamorphosis, we also found changes in the Kupffer cells, and in our opinion these provide evidence relating to the causes of appearance of pigmented cells in the liver and their function.

EXPERIMENTAL METHOD

Kupffer cell ultrastructure was studied in the liver of adult frogs (*Rana temporaria*) in the winter period, the tadpole liver in the period of metamorphosis, when the tail is undergoing resorption, and the liver of frogs in the first year of life. Pieces of liver were fixed in 2.5% glutaraldehyde in phosphate buffer and postfixed with 1% OSO_4 in the same buffer. Material was embedded in Epon. Ultrathin sections were stained by Reynolds' method and examined in the IEM-100C electron microscope.

EXPERIMENTAL RESULTS

Kupffer cells of hibernating adult frogs and in the liver of tadpoles with resorbed tail are much larger than in the adult frog liver in the summer period. The nucleus is elongated and the heterochromatin is located juxtamurally in a wide border. The cytoplasm contains a few cisterns of the rough endoplasmic reticulum. The mitochondria are small, and oval or elongated in shape. In the hibernating frog the mitochondria have a denser matrix than normally, and a widened intercristal space, whereas in the liver of tadpoles during resorption of the tail the structure of the mitochondria is normal and indistinguishable from that In the adult frog liver in the summer period. Sometimes a whole erythrocyte can be seen in the cytoplasm of the Kupffer cells; the peripheral part of its cytoplasm appears to consist of membranes, arranged parallel to one another along the whole perimeter, and resembling myelin (Fig. 1). A distinguishing feature of the majority of Kupffer cells studied is the large number of phagosomes, the large Golgi complex, and a mass of small cross sections of the smooth

Laboratory of Evolutionary Histology, A. N. Severtsov Institute of Evolutionary Morphology and Ecology of Animals, Academy of Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 1, pp. 82-84, January, 1992. Original article submitted July 1, 1991.