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Immunohistochemical Study of the Squamous Epithelium Antigen and the Possibility of Using It as a Marker of Squamous Cell Carcinoma

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With the use of polyclonal antibodies to the squamous epithelium antigen it is demonstrated that small amounts of this marker are expressed in the cytoplasm of some cells in the prickle-cell layer of the epithelium. The amount of this antigen increases in the parabasal layer of squamous epithelium with the severity of the dysplastic process. Study of 115 specimens of various histological types of tumors, shows that the specificity of the antibodies for the squamous epithelium antigen is 97.4% for squamous cell carcinomas. Thus, this antigen can be used for the identification of squamous cell carcinomas from nondifferentiated tumors.

Key Words: *squamous epithelium antigen; squamous cell carcinomas; immunohistochemical study*

An antigen originally identified as a protein associated with cervical squamous cell carcinoma was described previously [1,2]. Here we report immunohistochemical data on normal, dysplastically altered, and malignant tissues obtained with the use of antibodies to the squamous epithelium antigen (SEA). Our objective was to assess he possibility of using SEA for refining immunohistochemical diagnostics in oncology.

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MATERIALS AND METHODS

Tissue samples from 115 patients with tumors of various localization and histogenesis were obtained from the Surgery Department and the Pathomorphology Archive of th eP. A. Gertsen Institute of Oncology (Moscow). They were fixed in formalin and embedded in paraffin. Serial sections (4 μ thick) were deparaffinated by the standard method. The SEA was visualized by the indirect immunoperoxidase method. Endogenous peroxidase was inhibited with 0.03% H_2O_2 in methanol, and the sections were treated for 30 min at 37°C with anti-SEA antibodies (8-10 μg/ml), washed with

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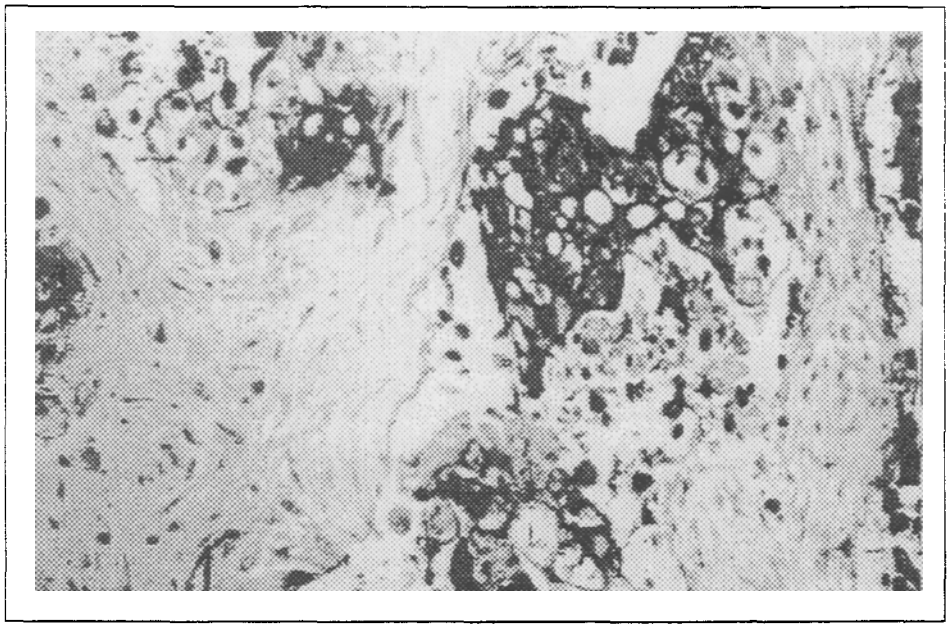

Fig. 1. Squamous cell lung carcinoma. The immunoperoxidase reaction reveals the squamous epithelium antigen in the cytoplasm of tumor cells. Counterstaining with hematoxylin. ×250.

phosphate-buffered saline (PBS, pH 7.4), and incubated with anti-rabbit immunoglobulins conjugated with horseradish peroxidase (1:32, N. F. Gamaleya Institute of Epidemiology and Microbiology, Moscow). A 0.05% solution of 3,3'-diaminobenzidine in PBS (pH 7.4) with 0.05% H_2O_2 was used as a substrate. After the reaction the sections were counterstained with hematoxylin. Nonimmune rabbit serum (1:100) and PBS (pH 7.4) were used as controls. Anti-SEA antibodies were isolated from rabbit anti-SEA antiserum absorbed by lyophilized extracts of normal human tissues [1,2], using an affinity sorbent: an extract of squamous cell carcinomas in 3 M KCl immobilized on BrCN-activated Sepharose-4B (Pharmacia) [2].

TABLE 1. Expression of SEA in Tumors of Different Organs

Localization	Number of cases	
	studied	positive
Squamous cell carcinomas		
Cervix	50	50
Esophagus	17	17
Larynx	2	2
Lung	9	7
Total (97.4%)	78	76
Other tumors		
Cervical adenocarcinoma	4	0
Breast, tracheal, colon, and ovarian cancer	4	1
Cancer:		
stomach	4*	1
endometrial	2	1
lung	10*	3
Total	30	6 (20%)

Note. An asterisk indicates that the reaction occurred in some cells.

RESULTS

The anti-SEA antibodies were tested in normal and pathologically altered cervical tissues. In normal squamous epithelium, the antigen was detected in the cytoplasm of some cells of the superficial and prickle-cell layers. The antigen was not detected in the columnar epithelium of the cervical canal. A weak positive reaction was observed in metaplastized squamous epithelium and in the reserve cells. The amount of the marker increased with the severity of the dysplastic process in squamous epithelium, particularly in highly differentiated intraepithelial carcinoma. The antigen was expressed in all specimens of cervical tumors diagnosed as squamous cell carcinoma in the areas of both low and high differentiation (Table 1). However, in the latter case large amounts of the antigen were revealed. Its most intense accumulation occurred in the foci of pronounced keratinization with formation of cancer "pearls." There was no specific SEA staining on the sections of cervical tumors morphologically diagnosed as adenocarcinoma.

Using anti-SEA antibodies, we also studied tumors of other localization and histogenesis (Tables 1 and 2; Fig. 1): squamous cell carcinomas with various degrees of differentiation, adenocarcinomas, mucoepithelioid, dimorphous and small-cell cancer, leiomyosarcoma, and carcinosarcoma. In squamous cell tumors the antigen was detected in the cytoplasm of tumor cells, as in

TABLE 2. Expression of SEA in Various Esophageal Tumors

Morphological type of tumor	Number of cases	Expression of SEA
Squamous cell carcinoma	17	Antigen expressed in tumor epithelium in large amounts at sites of keratinization with formation of "pearls." In squamous epithelium antigen present in cytoplasm of some cells.
Dimorphous cancer	4	Antigen detected in squamous component of tumor; in integumentary squamous epithelium it is detected in cytoplasm of some cells.
Carcinosarcoma	1	As above.
Adenocarcinoma	2	Small amounts of antigen present only in integumentary squamous epithelium.
Small-cell cancer	1	As above.
Leiomyosarcoma	1	—"—

squamous cervical carcinomas, its amount being greater in areas of higher differentiation. The reaction was much less intense in low-differentiated tumor cells. In highly differentiated keratinous cancers the antigen was accumulated predominantly in the cytoplasm of tumor cells which were in a state of parakeratosis. Thus, highly differentiated squamous carcinomas contained greater amounts of SEA than low-differentiated tumors. In cases of dimorphous or mucoepisquamous cell esophageal cancers, the antigen was revealed in the squamous component but was absent from the glandular component of the tumor.

In esophageal carcinoma, SEA was expressed only in the integumental squamous epithelium and was not detected in tumor cells. In tumors morphologically diagnosed as small-cell cancer and leiomyosarcoma, the antigen was detected only in the integumental squamous epithelium, tumor cells being antigen-negative. In esophageal carcinosarcomas, the epithelium component of which was represented by keratinous squamous cell carcinoma, the cytoplasm of tumor cells contained SEA deposits.

Thus, SEA is detected in the large majority of carcinomas with squamous cell differentiation. Antigens with similar characteristics have been described in a number of studies [3,5,6]. Clinical evaluation of SEA as a marker has been performed in some clinics and laboratories, and the results indicate that it is useful to determine its serum content for monitoring the course of squamous cell carcinoma of various localization (predominantly head, neck, and gynecological tumors, as well as neoplasms of the upper respiratory tract and alimentary canal) and for early diagnosis of recurrences [8-10]. Investigations of the tissue expression of SEA have made it possible to evaluate SEA as a marker which is a normal component of the mucosa and which reflects a certain level of cell differentiation [7]. There is evidence allowing for an optimistic interpretation of the expression of the antigen associated with pulmonary small-cell cancer, providing a statistically significant correlation between the presence of the antigen in the tumor and a favorable prognosis of the disease [4].

Our anti-SEA antibodies can be regarded as an experimental and diagnostic tool for subtyping malignant tumor cells and tissues with precancerous alterations. The expression of SEA is maximal in cells with a relatively high degree of differentiation. In this case both an increase and a decrease (for example, in low-differentiated cancer) in the degree of tissue maturation correlates with the level of expression of SEA. Consequently, like TA-4 antigen [7,8], SEA can be considered one of the markers of a certain level of cell differentiation and used with this characteristic taken into consideration. The presence of SEA in some cells of glandular tumors (stomach or lung cancer) can probably be regarded as a sign of double differentiation of cells in a neoplasm. This fact requires further investigation. Specifically, it can be useful for the development of prognostic criteria of the use of SEA as a marker of squamous cell differentiation.

We hope that the squamous epithelium antigen will be used as a marker of squamous epithelium together with other immunohistochemical tools of tumor diagnostics.

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Use of Glutathione Inhibitors and Glutathione Transferases to Overcome Tumor Resistance to Cytostatics *In Vivo*

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Preincubation of cells of BDF1 hybrid mice with P388 leukemia with doxorubicin and buthionine sulfoximine leads to the manifestation of a therapeutic effect of the antibiotic. Injection of buthionine sulfoximine and ethacrinic acid to mice with leukemia does not alter the therapeutic effect of the antibiotic.

Key Words: *glutathione; glutathione transferases; buthionine sulfoximine; ethacrinic acid; resistance*

The possible contribution of glutathione and glutathione-dependent enzymes to the maintenance of tumor cell resistance to cytostatics is being widely discussed at present. The levels of glutathione and glutathione-S-transferases have been shown to be increased in cells with multiple drug resistance phenotypes and in those resistant to alkylating agents [5,7]. In this connection the use of drugs inhibiting the activity of these enzymes and/or reducing the glutathione content is one of the methods of overcoming tumor cell resistance to cytostatics. Some reports advocate such an approach. However, these studies were carried out *in vitro*, and, hence, the possibility of using inhibitors of glutathione and glutathione transferases to overcome tumor resistance to cytostatics *in vivo* is still undocumented. The importance of such studies is self-evident, for obviously it is sometimes virtually impossible to attain *in vivo* the drug concentrations which are used *in vitro*.

We investigated the effects of buthionine sulfoximine (BSO), a glutathione-S-transferase inhibitor, and ethacrinic acid (EA), a glutathione inhibitor, on the abolishment of doxorubicin (DX) resistance of mouse leukemia P388 with induced antibiotic resistance (P388/DX). Previously we demonstrated a 3.5-fold increase of glutathione transferase activity in P388/DX leukemia cells in comparison with the initial sensitive strain, this permitting us to use this tumor strain as a model [1].

MATERIALS AND METHODS

Experiments were carried out with hybrid BDF1 mice aged 2 to 3 months. P388 leukemia cells sensitive to DX (P388/0, tumor strain bank of the Cancer Research Center, Russian Academy of

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