Metastasizing of Human Melanoma on Immunodeficient Mice. Comparison of Cell Lines with Different Metastasizing Activity

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Two human melanoma cell variants - with low and high metastasizing activity - are obtained by successive passaging on mice with combined immunodeficiency. After the development of a subcutaneous tumor, tumor cells are detected only in the bloodstream of animals with a highly metastasizing tumor, in mice with combined immunodeficiency the number of these cells being much greater than that in nude mice. These results indicate a preeminent influence of the nature of tumor cells on the dissemination of metastasizing cells.

Key Words: *metastasizing; melanoma; blood; nude mice; beige/nude mice*

Evidence confirming the dissemination of human tumor cells in the bloodstream of mice having been obtained [1], it is reasonable to speculate upon a possible relationship between dissemination and the metastasizing activity of these cells. Some morphological and immunochemical comparisons of selectively obtained tumors with high and low metastasizing activities have been reported [4]. It is necessary to determine which factor is more important for dissemination and, consequently, for metastasizing: species-specific interlinear genetic differences of animals, i.e., genetically determined immunological status, or genetically determined differences between tumor cell variants.

MATERIALS AND METHODS

A human melanoma cell line (MEL-7) was obtained at the Laboratory of Experimental Models (Cancer Research Center, Russian Academy of Medical Sciences) from the Bro strain [5] by successive selection for the ability to metastasize in the lungs and was divided into two sublines with low and high metastasizing potential. Experiments were performed on 7-week-old immunodeficient mice (thymus-free nude mice and beige/nude hybrids) bred in the laboratory. Cell suspensions (10^7 cells per injection) were injected subcutaneously in the back. Blood (0.5 ml) was collected from the caudal vein on days 20-27 in a mixture of the blood-preserving solution Glugicyr and normal saline (1:4). The cells were stained with FITC-conjugated monoclonal antibodies to the tumor-associated antigen of human melanoma and analyzed in an EP-ICS V cytofluorimeter (Coultronics) equipped with an Innova 90 argon laser (Coherent). At least 10^5 cells were analyzed in each experiment.

RESULTS

The mean fluorescence of circulating cells with a high metastasizing activity was significantly higher than that of cells with a low activity both in mice

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TABLE 1. Logarithm (Relative Units) of Mean Intensity of Fluorescence of Human Melanoma Cells (MEL–7) in Peripheral Blood of Nude and Beige/Nude Mice

Autofluorescence	Line			
	nude		beige/nude	
	high-metastasizing	low-metastasizing	low-metastasizing	high-metastasizing
57.78	63.71	66.41	63.61	71.24
	63.24	66.34	63.84	71.64
	62.05	65.89	63.97	72.03
		67.07		71.75
		66.69		74.35
				72.05
mean	63.00	66.48	63.81	72.25

with inherited T-cell immunodeficiency (nude) and in mice with inherited combined immunodeficiency (beige/nude) (Table 1).

It can be seen from the table that the fluorescence intensity is the highest in hybrid (beige/nude) mice with highly metastasizing melanoma. This trend becomes evident after analyzing the individual amounts of the antigen-carrying cells in peripheral blood samples (Table 2).

These findings indicate that the blood of both nude and beige/nude mice carrying low metastasizing tumor cells contains only trace amounts of antigen-positive cells, whereas in mice carrying highly metastasizing cells the amounts of antigen-positive cells in some cases can be much higher that those observed with low-metastasizing cells in nude and, particularly, in beige-nude mice. This points to the fact that in this model metastasizing depends on the properties of a cell line rather than on the status of the immune system. However, the maximum dissemination of highly metastasizing cells in the bloodstream of mice with combined immunodeficiency probably indicates an indirect involvement of the immunological control systems.

Our results agree with published data. Experiments with human tumor cells exhibiting different metastasizing activity performed on nude mice and mice with combined immunodeficiency (beige/nude hybrids and analogous hybrids) have shown that the ability to produce metastases is a cell property which depends on (or is accompanied by) a number of factors such as the expression of specific receptors structurally similar to transferrin [8], integrins [2], various tumor-associated antigens [9], the lipid composition of the tumor cell plasma membrane [3], the glycoconjugate profile of cells, and their enzyme activity [6]. Obviously, not all these factors play a key role; we did not find differences in the binding of visualizing monoclonal antibodies to the different cell variants and, consequently, with the expression of a tumor-associated antigen.

TABLE 2. Number of Antigen–Positive Cells $\times 10^5$ in the Peripheral Blood at a Chosen Discriminating Value

Line			
nude		beige/nude	
low-metastizing	high-metastizing	low-metastizing	high-metastizing
0	324	0	5241
0	311	0	5320
0	274	0	5471
0	449	0	5380
0	318	0	5490
			5317

The finding that the number of cells with high metastasizing activity in the peripheral blood of beige/nude mice is considerably higher than in nude mice is consistent with the results of others [10]. Studies of several highly metastasizing human tumor cell lines have shown that metastases are produced both in nude mice and in mice with combined immunodeficiency, the number of these cells in the latter being 3 times as high as in the former [10].

Nevertheless, our data on the prediction of the significance of the properties of a tumor cell in the process of metastasizing do not indicate the equal suitability of nude and beige/nude mice for investigations in the field of modeled metastasizing. Obviously, the results obtained on mice with combined immunodeficiency are more demonstrative, contrasting, and easier to record. This is consistent with the idea that the animals with combined immunodeficiency are more suitable for the generation of tumor cell lines with a high metastasizing activity [8].

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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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