Comparative Study of Metastases of Human Melanoma Strain Mel-7 in Nude and Beige/Nude Mice

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Metastases to the lungs after subcutaneous transplantation of human melanoma Mel-7 to nude and beige/nude mice are situated deep inside the organ closer to the root. Despite a larger size of xenograft in nude mice, the metastasizing process was more expressed in beige/nude mice with a more severe immunodeficiency, which was reflected in an increased number of metastases and their greater total volume in the lungs.

Key Words: *metastases; xenograft; nude mice*

Spontaneous metastases to the viscera are rarely observed after transplantation of human malignant tumors to nude mice [4]. Such a disparity between the tumor dissemination process and clinical data has led to the use of animals with more profound immunodeficiencies (beige/nude, SCID, etc.) in recent years. This yielded a stable model system with a high (in comparison with nude mice) level of spontaneous metastasis after transplantation of human tumors to these animals [2,6], although such a correlation was sometimes not observed [2].

Virtually none of the 65 strains of human tumors of different origin in our collection produced spontaneous metastases when transplanted subcutaneously to nude mice, there being just a few exceptions [5]. In this study we compared spontaneous metastases of human melanoma in immunodeficient nude and beige/nude mice.

MATERIALS AND METHODS

Experiments were carried out with T-cell deficient nude thymus-free mice bred on the basis of BALB/c mice and T-cell- and normal killer-deficient beige/nude males and females aged 1.5 months at the Department of Experimental Tumor Models, Cancer Research Center, Russian Academy of Medical Sciences. The Mel-7 strain of pigment-free human melanoma was obtained as a result of multiple serial passages by subcutaneous injections of Bro cell culture to nude mice [3]. The Mel-7 strain was transplanted subcutaneously to nude and beige/nude mice in the right scapular area by a routine method. Animals with tumors were sacrificed after 19-40 days when they were in a premorbid state. Tumor volume was assessed from the amount of fluid expressed. The lungs, liver, kidneys, spleen, and mediastinal lymph nodes together with the rudiments of the thymic stroma were fixed in 10% neutral formalin. Slices 7 μ thick were stained with hematoxylin-eosin. A hundred slices per animal were made for estimating the total number of metastases to the lungs. The volume of spherical metastases was estimated by the formula $V=\pi/6D^3$, and that of metastases with a displaced center by the formula: $V=\pi Q^2/6D^3$, where Q is a coefficient showing the degree of deviation of metastasis shape from the spherical (from 0.03 to 1).

RESULTS

Examination of the surface of organs before and after fixation revealed no metastases in any of the cases, including lung specimens stained with toluidine blue. Histologic examination of the liver, kidneys, and spleen

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TABLE 1. Incidence and Volume of Metastases to the Lungs in Nude and Beige/Nude Mice Transplanted Mel-7 Melanoma Strain (*M±m*)

Parameter	Mice	
	nude	beige/nude
Volume of tumor graft, cm^3	4.6±2.8 (2.5-6.7)	1.2±0.5** (0.9-1.4)
Time after transplantation, days	33.4±2.0 (24-40)	33.8±4.1 (19-40)
Percentage of mice with metastases	27.3 (3 out of 11)	45.5 (5 out of 11)
Mean number of metastases per mouse	29.2±8.8 (12-40)	53.8±11.0* (23-97)
Mean volume of a metastasis, mm^3	0.005 (0.001-0.051)	0.007 (0.002-1.214)
Mean volume of all metastases per mouse, mm^3	0.133 (0.056-0.220)	1.589 (0.04-6.516)

Note. *$p>0.05$, **$p<0.01$.

revealed no metastases either. In the lungs metastases were found inside the tissue, with rare exceptions. Under the pleura only 5 very small (less than 0.02 mm in diameter) metastases were found in all the animals. The location of metastases was the same in all mice, irrespective of the level of immunodeficiency: close to the root of the lung. The overwhelming majority of the metastases were very small (0.04 to 0.1 mm in diameter), although larger tumors, up to 2 mm long, were sometimes found directly at the root of the lung. All the largest metastases without exception were found in beige/nude mice. The metastases varied in shape: round, ellipsoid, finger-shaped, etc. Slices of the root of the lung, where not only the most numerous, but also the largest metastases were found, were the most representative for assessing the metastasizing process. Minor lymphocytes were often observed in the metastases, particularly in nude mice, and just a few polynuclears. The color of the tumor cells varied from sharply basophilic to weakly oxyphilic.

The data indicate that nude mice develop tumors that are several times larger after subcutaneous injection of the Mel-7 strain than do beige/nude mice, despite the same mean period of observation (Table 1). The level of metastases and mean number thereof per mouse were higher in beige/nude than in nude mice. The mean volume of a metastasis was the same in both groups, although the largest metastases were found in beige/nude mice. The mean volume of all metastases per animal was definitely higher in beige/nude mice, that is, the extent of lung tissue involvement in them was appreciably higher than in nude mice.

The results of this study of spontaneous metastasizing of Mel-7 melanoma demonstrate that metastases are virtually never found under the pleura in either immunodeficient mouse strain, as is the case in regular mice transplanted melanoma B16 and many other murine metastasizing tumors [1]. The metastases are situated deep inside the organ close to its root. The level of metastasizing depended on the degree of genetically determined immunodeficiency and was higher in beige/nude than in nude mice. Human melanoma Mel-7 may thus serve as a model for studying the metastasizing processes in animals with different degrees of immunodeficiency.

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