Metastatic Phenotype of Human Melanoma Substrain Mel-7m Grafted Into Immunodeficient Mice

I. P. Bryzgalov, Yu. D. Sorokina, V. V. Deev, T. E. Kaminskaya, E. S. Revazova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 122, No. 12, pp. 655-657, December, 1996
Original article submitted October 25, 1995

Metastatic phenotype of a new stable human melanoma substrain (Mel-7m) is described. After grafting into beige/nude mice, these cells spontaneously metastasize into the lungs in 84.2% of cases. The strain can be used in experimental oncology.

Key Words: *metastasizing; xenograft; nude mice; beige/nude mice*

Thymus-free mice are a convenient model for the investigation of transplantable human tumors [4]. However, after subcutaneous grafting into these mice, malignant tumors rarely produce metastases into internal organs [3,5]. This imposes certain restrictions on the use of immunodeficient mice for the investigation of metastasizing and for estimation of systemic antidisseminative therapies. This problem can be solved with the use of human tumor cells exhibiting stable metastasizing activity in immunodeficient mice.

Previously, we showed that human melanoma cells Mel-7 spontaneously metastasize after subcutaneous grafting into nude and beige/nude mice [1]. Metastases were revealed only in the lungs in 27% of nude mice and in 45% of beige/nude mice. In order to change the metastasizing phenotype of Mel-7 cells, lung metastases produced by these cells were grafted into nude and beige/mice and serially passaged through them. The metastasizing activity of a new substrain (Mel-7m) was then assessed.

MATERIALS AND METHODS

Experiments were performed on nude and beige/nude mice of both sexes aged 1.5 months. The mice were bred at the Laboratory of Tumor Models (Oncology Research Center, Russian Academy of Medical Sciences).

The mice were tested for the natural killer cell deficiency using NK-sensitive YAC-1 cells.

Pigment-free human melanoma cell line Mel-7m was obtained by subcutaneous grafting of Mel-7 into thymus-free mice with subsequent serial passaging of lung metastases in beige/nude mice.

Mice with tumors were killed in the premorbid state. Lungs, liver, kidneys, spleen, and mediastinal lymph nodes were collected and fixed in 10% neutral formalin. Sections (7 μ) were prepared and stained with hematoxylin and eosin. Metastases were counted on 100 sections cut though the entire organ. The volume of spherical metastases was calculated from the formula: $V=\pi/6D^3$ and the volume of metastases with a displaced center from the formula: $V=\pi Q^2/6D^3$, where Q (0.03-1.0) is the coefficient reflecting the extent to which the shape of a metastasis differs from spherical.

RESULTS

Visual examination of internal organs before and after fixation with formalin revealed no surface metastases. There were no metastases in the liver, kidneys, and spleen, as evidenced by histologic analysis. In two beige/nude mice metastases were found in the mediastinal lymph nodes. Histologic examination revealed occasional very small (<0.02 mm in dia-

Oncology Research Center, Russian Academy of Medical Sciences, Moscow

0007-4888/96/0012-1217$15.00 ©1997 Plenum Publishing Corporation

TABLE 1. Occurrence and Volume of Lung Metastases in Nude and Beige/Nude Mice After Grafting of Mel-7m Human Melanoma Substrain

Parameter	nude/nude	beige/beige/nude/nude
Mean volume of xenograft, cm^3	5.4±1.1 (1.9-8.8)	2.7±0.6 (0.9-6.5)
Mean survival after tumor grafting, days	29.4±1.3 (14-41)	23.4±1.4 (13-40)
Percent of mice with metastases	29.6 (8/27)*	84.2 (16/19)*
Mean number of metastases per mouse	25.0±4.0 (10-51)	95.0±10.2 (10-152)
Mean volume of one metastasis, mm^3	0.015 (0.001-0.690)	0.024 (0.001-0.753)
Mean volume of all metastases per mouse, mm^3	0.407 (0.044-2.792)	1.856 (0.012-7.528)

Note. Variation range is given in parentheses. *Number of animals with metastases to number of animals in the group.

meter) metastases under the pleura. The majority of the metastases (0.05-0.1 mm in diameter) were located at the radix of the lung. Occasional large metastases (1-2 mm in diameter) were found predominantly in beige/nude mice. After the same time period, the diameter of the xenograft in nude mice was twice that as in beige/nude mice (Table 1). In both mouse strains, the number of metastases strongly depended on the duration of tumor growth, while the relationship between metastasizing and tumor volume was weaker.

The percentage of metastases formed in mice with double immunodeficiency were 2.8-fold higher than in thymus-free mice ($p \leq 0.05$).

The boarders for the distribution of metastases according to their volume were almost the same in both groups. However, since in beige/nude mice the number of metastases was greater and the occurrence of large metastases was higher (which is reflected by the mean volume of one metastasis), the proportion of tumor tissue in the lung (mean volume of all metastases per mouse) was 4.6-fold greater than that in nude/nude mice.

These findings show that the metastasizing activity of Mel-7m cells was considerably higher in mice with double immunodeficiency. Similar results were obtained with Mel-7 cells [1]. However, in experiments with Mel-7m cells, the occurrence of lung metastases was higher in beige/nude mice than in nude mice. Bearing in mind that nude and beige/nude mice are genetically different and melanoma cells were passaged in beige/nude mice, it can be suggested that the affinity of these cells for pulmonary tissue increased only in beige/nude mice.

However, it was demonstrated that after subcutaneous grafting, the quantities of Mel-7 cells circulating in peripheral blood are very low both in nude and in beige/nude mice. After subcutaneous grafting of Mel-7m cells, the quantities of circulating cells were higher in both mouse strains (compared with Mel-7 cells). In beige/nude mice, blood content of Mel-7m cells was higher than in nude mice [2].

The results of the present study are consistent with our previous findings, indicating that some factors produced in the body of nude mice either prevent the entry of tumor cells into peripheral blood, or, which seems more probable, induce lysis of tumor cells in the bloodstream involving natural killer cells. Beige/nude mice are a preferable host for Mel-7 and Mel-7m human melanoma cells in investigation of metastasizing processes.

REFERENCES

1. I. P. Bryzgalov, Yu. D. Sorokina, Yu. N. Solov'ev, and E. S. Revazova, *Byull. Eksp. Biol. Med.*, **121**, No. 1, 89-90 (1996).
2. A. D. Mikhailov, A. A. Malakhov, E. S. Revazova, *et al.*, *Ibid.*, **119**, No. 2, 206-208 (1995)
3. J. M. Kozlowski, I. J. Fidler, D. Campbell, *et al.*, *Cancer Res.*, **44**, 3522-3529 (1984).
4. J. Rygaard and C. O. Povlsen, *Acta Path. Microbiol. Scand.*, **77**, 758-760 (1969).
5. E. L. Wilson, M. F. R. M. Gartner, J. A. H. Campbell, and E. B. Dowdle, *Int. J. Cancer*, **41**, 83-86 (1988).