ONCOLOGY

Specificity of Relapses and Metastases of Experimental Transplanted Ehrlich Carcinoma and B16 Melanoma

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 143, No. 1, pp. 86-88, January, 2007
Original article submitted June 21, 2006

Experiments on female (CBA×C57Bl/6)F_1 mice with simultaneously transplanted Ehrlich carcinoma and B16 melanoma showed that removal of one of the primary nodes led to metastasizing of the removed tumor alone. It seems that this specificity of the inhibitory effect of the primary tumor can be explained by peculiarities of glycosylation and subsequent complex formation of serum proteins with the tumor.

Key Words: *relapses; metastases; Ehrlich carcinoma; B16 melanoma*

The phenomenon of metastases development after removal of the primary tumor node was described not once [1,7,8], but its mechanism remains unknown. Presumably, stimulation of the metastatic process after tumor elimination is associated with the release of angiogenic factors. Stimulation of vessel formation improves tumor trophics and hence, promotes its growth. Induction of metastases after removal of the transplanted tumor was shown on experimental models [9,10]. This effect was attributed to inhibition of the production of angiogenic factor in metastases by the primary node. Similar results were obtained in comparative study of vascularization of synchronous metastases of colorectal cancer into the liver in patients before and after removal of the primary tumor node [9,10]. Attempts at isolation of the primary tumor inhibitory factor failed and its existence was not confirmed [7]. Moreover, the problem of specificity of angiogenic factors for each tumor remains unsolved [3].

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We studied the specificity of metastases of tumors of different origin after removal of the primary node.

MATERIALS AND METHODS

Experiments were carried out on female (CBA×C57Bl/6)F_1 hybrid mice. Ehrlich carcinoma was transplanted into the thigh muscles (10^6 cells in 0.1 ml medium 199). B16 melanoma was transplanted subcutaneously (10^6 cells in 0.1 ml medium 199) on day 10 after transplantation of Ehrlich carcinoma. The primary node was resected under ether narcosis. Ehrlich carcinoma was removed on day 25, B16 melanoma on day 15 after transplantation. The mice were sacrificed after the death of the first animal in the group (on day 35 after Ehrlich carcinoma, on average). Each group consisted of 10 animals.

In order to evaluate the relationship between the removal of B16 melanoma primary node and the life span, the mice were operated on day 15 after tumor transplantation. The mean life span in comparison with non-operated animals served as the

0007-4888/07/1431-0080

criterion for evaluating the results. The data were processed using the Fisher—Student test. The differences were significant at $p<0.05$.

RESULTS

Surgical removal of the tumor node did not change the life span of mice with transplanted Ehrlich carcinoma. In non-operated mice, the tumor grew into the abdominal cavity; no metastases were detected in this group. After the operation 40-60% mice developed relapses. Irrespective of this process, all animals developed metastases into the abdominal lymph nodes and spleen. Metastatic growth in the subcutaneous fat was noted in 20-30% cases.

Comparative analysis of the life span of animals with transplanted B16 melanoma after surgical resection of the tumor node showed similar results. The mean life span of non-operated animals was 36.2±2.0 days *vs.* 39.4±2.1 days in operated mice; hence, tumor resection did not lead to an appreciable prolongation of the life span. In non-operated mice tumor growth into the abdominal cavity was observed, but no metastases were found. About 40% mice had relapses after the operation; no metastases in the lungs were detected in these animals. Metastatic growth in the subcutaneous fat

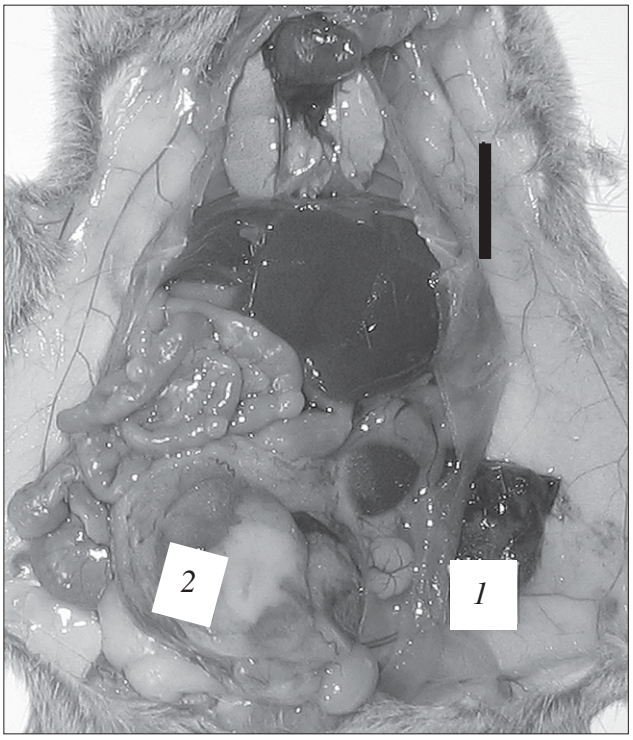

Fig. 1. Ehrlich carcinoma and B16 melanoma relapses and metastases in mice with resected primary Ehrlich carcinoma node. *1*) B16 melanoma (primary tumor); *2*) Ehrlich carcinoma metastasis. Here and in Figs. 2, 3: scale: 1 cm.

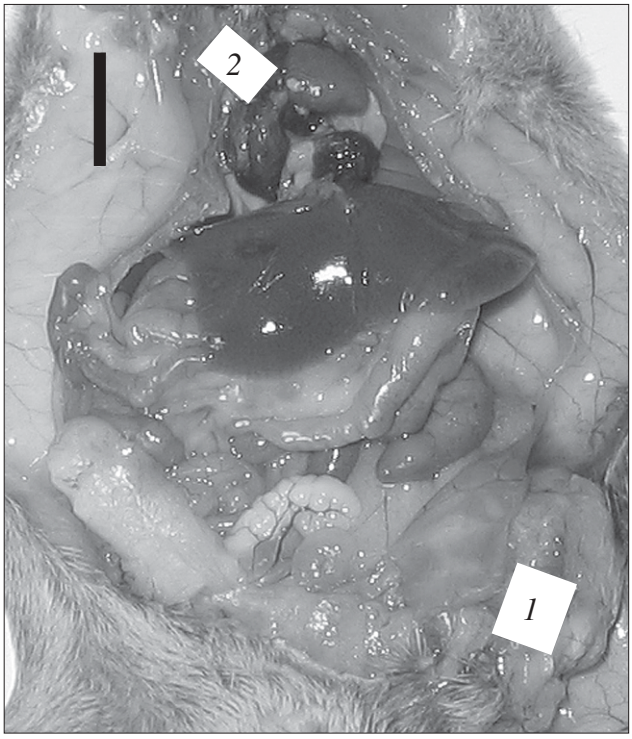

Fig. 2. Ehrlich carcinoma and B16 melanoma relapses and metastases in mice with resected primary B16 melanoma. *1*) Ehrlich carcinoma (primary node); *2*) B16 melanoma metastases.

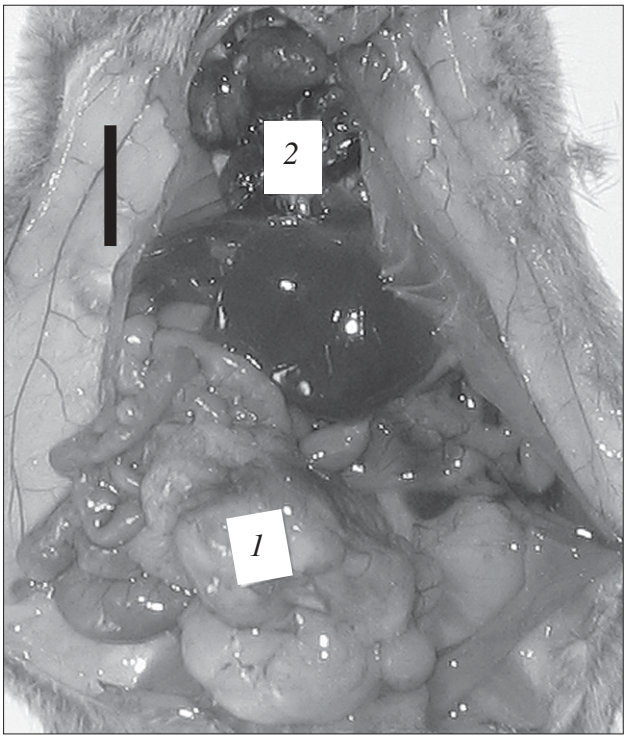

Fig. 3. Ehrlich carcinoma and B16 melanoma relapses and metastases in mice with both primary tumors resected. *1*) Ehrlich carcinoma metastasis; *2*) B16 melanoma metastases.

was detected in 20-30% cases, which can be caused by microtrauma of these sites of subcutaneous fat (clamping during the operation or cicatrization).

After removal of Ehrlich carcinoma in mice with two transplanted tumors, metastases into the abdominal lymph nodes and into the spleen (only Ehrlich carcinoma) developed (Fig. 1). No B16 melanoma metastases were detected. Removal of B16 melanoma was followed by the development of metastases of this tumor in the lungs, but not Ehrlich carcinoma metastases in the abdominal cavity lymph nodes and spleen (Fig. 2). Removal of both primary nodes led to the development of Ehrlich carcinoma metastases in the abdominal cavity and of B16 melanoma in the lungs (Fig. 3).

Hence, the mean life span of non-operated animals and mice after tumor removal was virtually the same. Presumably, the life span of animals after the operation was not prolonged because of more rapid metastatic growth, due to disappearance of the inhibitory effect on the angiogenic factors of the primary tumor. Metastatic growth of only resected tumor was observed in animals with two transplanted tumors (Ehrlich carcinoma and B16 melanoma); the life span was determined by the growth rate of the more rapidly growing tumor (B16 melanoma), this indicating some specific effect. This specific effect cannot be mediated by the known angiogenic factors [2,3,6], because they should be common for all tumors and stimulate metastatic growth of both Ehrlich carcinoma and B16 melanoma after resection of one of the tumors. We think that the specific inhibitory effect of the primary tumor can be due to peculiar glycosylation and subsequent formation of complexes of serum proteins with the tumor [3-5]. The formation of complexes with serum proteins (the complex consists of 4 proteins, one of which is haptoglobin with 20% carbohydrates) was detected in mice with Ehrlich carcinoma.

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