FREQUENCY, TIMES, AND TYPE OF METASTASIZATION OF TRANSPLANTABLE TUMORS IN MICE

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The character of metastasization of nine strains of transplantable mouse tumors injected by the usual subcutaneous method was studied. Differences were observed in the frequency, intensity, and types of metastasization of different tumors. Latent periods of onset of metastases of Lewis and RL-67 lung carcinomas and of sarcoma-37 were determined. Sarcoma-37, Lewis and RL-67 carcinomas of the lung, AKATOL adenocarcinoma of the large intestine, and Cloudman's and B-16 melanomas metastasized most intensively. Sarcoma-37, metastasizing into the lungs, RL-67 lung carcinoma metastasizing into the lungs, kidneys, adrenals, ovaries, and heart, and adenocarcinoma of the large intestine AKATOL, forming metastases in the lymph nodes and liver, can be used as models for seeking methods of chemotherapeutic action on metastases and metastasization.

KEY WORDS: transplantable tumors; metastasization.

In experimental investigations to seek methods of pharmacological action on metastasization various models and methods are used to obtain metastases. Many such investigations have been carried out on animals with intravenous inoculation of tumor cells [8-12]. However, several workers have shown that models with intravenous inoculation of tumor cells have a limited field of use because of their degree of artificiality. In the present writers' opinion, models with subcutaneous inoculation of the tumor into the tail [1-3, 5-7], also are unsuitable, for tumors which do not metastasize or do so to a limited degree when inoculated by the usual method (subcutaneous or intramuscular), when inoculated by this method give a high percentage of metastasization.

Recent work by Soviet investigators [4] have shown that transplantable tumors of animals metastasize in the same way as corresponding human tumors, and that evidently they can serve as a convenient model of the latter when studying problems connected with metastasization of tumors and the development of schemes for the treatment of metastases.

The object of this investigation was to assess any special features of metastasization of various transplantable mouse tumors and to select the most suitable models for later investigations in the chemotherapy of metastases of tumors.

EXPERIMENTAL METHOD

Experiments were carried out on nine different strains of mouse tumors (sarcoma-37, adenocarcinoma of the mammary gland AK-755, adenocarcinoma of the large intestine AKATOL, Lewis and RL-67 carcinomas of the lung, B-16, Cloudman's and Harding-Passy melanomas, and carcinoma of the cervix uteri RShM-5). Tumors were taken from the bank of tumor strains, Oncologic Scientific Center, Academy of Medical Sciences of the USSR, were passaged twice through donor animals, and were then reinoculated for the experimental study. For reinoculation the tumor tissue was cut into small pieces with scissors to a homogeneous consistency, diluted with medium No. 199 in the ratio of 1 : 10, and 0.5 ml of the resulting suspension was inoculated subcutaneously into the region of the axilla. Sarcoma-37 was transplanted into SHY/KV mice, Lewis and RL-67 carcinomas of the lung, adenocarcinoma AK-755, and the Harding-Passy, B-16, and Cloudman's melanomas

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TABLE 1. Frequency of Metastasization and Predominant Localization of Metastases of Some Transplantable Mouse Tumors

Strain of tumor	Recipients	Sex	Mean life-span (days)	Metastasization		
				type	frequency in %	predominant location of metastases
Sarcoma-37	SHY/Kv	♂	52.0	Mainly lymphogenous	100	Peripheral lymph nodes, thymus, adrenals
Adenocarcinoma of large intestine AKATOL	BALB	♂	29.0	Mixed	100	Peripheral lymph nodes, liver
Lewis carcinoma of lung	BDF_1	♂	24.0	Hematogenous	100	Lungs
RL-67 carcinoma of lung	BDF_1	♀	36.0	Hematogenous	100	Lungs, kidneys, heart, ovaries, adrenals
B-16 melanoma	BDF_1	♂	27.0	Hematogenous	60-90	Lungs
Harding-Passy melanoma	BDF_1	♂	73.0	Lymphogenous	35.0	Peripheral lymph nodes
Cloudman's melanoma	BDF_1	♂	47.0	Hematogenous	75.0	Lungs
RShM-5 carcinoma of cervix uteri	CBA	♀	40.0	Mainly lymphogenous	55.0	Peripheral lymph nodes
AK-755	BDF_1	♀	26.0	–	–	–

into first-generation hybrid (BDF_1(C57BL × CBA/2) mice, adenocarcinoma of the large intestine AKATOL into BALB/C mice, and carcinoma of the cervix uteri RShM-5 into CBA mice. The experiment with RL-67, RShM-5, and AK-755, were carried out on females, all the rest on males. The animals used in the experiments were of the same age and of about the same weight. The autopsy material was examined only macroscopically. No metastases were found visually in the organs.

To determine the times of appearance of metastases the method used involved removal of the primary tumor nodule at various times after inoculation, and its essential details are as follows. The tumor was transplanted as described above. At various time intervals after transplantation (on the 3rd-9th day for Lewis and RL-67 carcinomas and on the 3rd-14th day for sarcoma-37) the primary tumor with the adjacent area of skin, sufficiently large in area to prevent the possibility of recurrence, was removed surgically under ether anesthesia. In the experiment with sarcoma-37, which metastasizes mainly by the lymphogenous route, the regional lymph nodes (inguinal and axillary) were preserved, whereas in the other two cases they were removed. After removal of the tumor the skin defect was closed with sutures. The animals tolerated both the anesthetic and the operative trauma well. After a period of time equal to the mean life span of the control animals with tumors, the experimental animals from which the primary tumor nodules had been removed were killed and subjected to macroscopic examination. If metastasization in the organs had begun to take place before removal of the primary tumor, developed metastases were found at autopsy.

TABLE 2. Times of Metastasization of Some Transplantable Mouse Tumors

Strain of tumor	Time of removal of primary tumor (days after inoculation)	Presence of metastases (ratio of number of mice with metastases to total number of mice in group)
Lewis carcinoma of lung	3	0/8
	5	1/8
	7	7/8
	9	8/8
RL-67 carcinoma of lung	3	0/8
	5	0/8
	7	7/8
	9	8/8
Sarcoma-37	3	0/10
	5	0/10
	7	0/10
	9	0/10
	12	0/10
	14	2/10

EXPERIMENTAL RESULTS

As Table 1 shows, sarcoma-37, Lewis and RL-67 carcinomas of the lung, and adenocarcinoma of the large intestine AKATOL metastasized most intensively. In the animals which died metastases were found in 100% of cases. All three strains of melanoma metastasized less intensively. Adenocarcinoma AK-755 virtually did not metastasize. Differences in the type of metastasization and location of the metastases were found. For instance, sarcoma-37 metastasized mainly by the lymphogenous route. Metastases of considerable size were found in the axillary, inguinal, retroperitoneal, paratracheal, and submandibular lymph nodes as well as, in isolated cases, the adrenals, lungs, and thymus. The lymph nodes (inguinal, axillary, submandibular) both on the side of inoculation of the tumor and on the opposite side, incidentally, were intensively involved in the process. Lewis and RL-67 carcinomas and also Cloudman's and B-16 melanomas metastasized by the hematogenous route. The only place where macroscopically visible metastases of the above tumors (except RL-67) were observed was the lungs. Carcinoma RL-67 also metastasized in the heart, kidneys, ovaries, and adrenals. Adenocarcinoma of the large intestine AKATOL metastasized in a mixed manner. In 100% of animals visible metastases were found in various groups of lymph nodes, and in 60% of animals in the liver also. This tumor acquires its ability to form metastases in the liver in the course of serial passages. Initially adenocarcinoma of the large intestine metastasized only by the lymphogenous route to the regional and distant lymph nodes.

The results of the experiments to determine the times of metastasization are given in Table 2. They show that metastasization of Lewis and RL-67 carcinomas in the lungs began between the 5th and 7th days. If the primary tumor was removed on the 7th day, tumor cells were already present in the lungs in sufficient numbers for metastases to develop later. However, in no case were metastases of carcinoma RL-67 found in organs such as the kidneys, heart, or ovaries, even if the primary tumor was removed on the 9th day after inoculation. In the experiments with sarcoma-37 metastases were found in only 2 of 10 cases in the regional lymph nodes on the side of inoculation of the tumor if the primary tumor was removed on the 14th day after transplantation.

Metastasization of tumors is a long-term process and the fate of the metastasizing cells at each stage of this process largely depends on relations between the host organism and the tumor growing in it. Models in which metastases developed "spontaneously," against the background of a progressively growing tumor, must therefore be regarded as more adequately reflecting the natural course of the process than models based on dissemination of tumor cells injected into the blood stream of an intact animal. It could be concluded from this study of the character of metastasization of a number of transplantable mouse tumors that the most suitable of them for experimental research on the chemotherapy of metastases are Lewis and RL-67 carcinomas, adenocarcinoma of the large intestine AKATOL, sarcoma-37, and Cloudman's and B-16 melanomas. The high frequency and intensity of metastasization characteristic of these models enables the effectiveness of a particular method of chemotherapeutic action on metastases and metastasization to be assessed quantitatively, while the information obtained on the times of appearance of metastases is essential for planning schemes of treatment of metastases.

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