THE METASTASIZATION OF RAT KIDNEY CARCINOMA RA

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The study of distribution and localization of metastases in an organism is of great significance in experimental and clinical oncology. Of special interest are studies on malignant cells carried by the blood stream and entering into organs, without giving rise to macroscopically visible metastases [3, 7].

The most controversial and important question is that pertaining to the viability of malignant cells circulating throughout the bloodstream of cancer patients. Some authors [5, 6] consider that cancer cells do not lose their invasive properties after being carried by blood flow, while others [4, 8] on the contrary believe that most of the malignant cells, which enter into the bloodstream, die.

In this investigation our purpose was to elucidate the ability to metastasize of the relatively rare rat epithelial tumor (cancer of the kidney) and the viability of the malignant cells of this tumor present in the circulating blood and in organs of animals, in the absence of macroscopically visible metastases.

RESULTS

Three to six months old Wistar rats of both sexes, injected with rat kidney carcinoma RA [1] strain were used in this work. The tumor tissue was injected subcutaneously, into the testes, spleen, liver, lungs, kidney, tongue,and tail. In subcutaneous injections the tumor tissue was injected in the region of the hind leg in a dose of 1 ml of a 10% suspension of tumor fragments in saline and in injections into the other above mentioned organs, in doses of 0.2-0.5 ml of the same suspension.

TABLE 1. Distribution and Localization of Metastases of Kidney Carcinoma RA in Rats, Following Injection of the Tumor Tissue into Different Organs.

Site of injection	no. of animals	Metastases in									
		liver	kidneys	spleen	lungs	stomach	intestine	diaphragm	greater and lesser omentum	peritoneum	lymph nodes
Subcutaneous	50	0	0	0	0	0	0	0	0	0	0
Testes	70	0	0	0	0	0	0	0	70	70	70
Spleen	15	12	0	—	0	3	4	2	15	15	15
Kidney	15	0	0	0	0	0	0	0	15	15	15
Liver	15	0	0	0	0	0	0	0	15	15	15
Lungs	6	0	0	0	0	0	0	0	0	0	0
Tongue	6	0	0	0	0	0	0	0	0	0	0
Tail	16	0	0	0	0	0	0	0	0	0	0

TABLE 2. Results of Injection into Normal Rats of Whole Blood and of Organ Suspensions from Animals Suffering from Carcinoma

Material injected	Injections		Latent period of tumor growth (in days)	Period of survival of animals following injection (in days)
	total no.	no. positive		
Organs of rats with metastasizing tumor	20	11	10—12	25—35
Organs of rats with subcutaneously injected tumor	32	0	—	—
Blood of animals with tumors	25	3	10—12	25—35

The presence and viability of malignant cells circulating in blood and located in internal organs of animals suffering from carcinoma were determined biologically i.e, by means of subcutaneous injection into normal rats. For this purpose two-months-old rats were used. Kidney, liver, spleen, and lungs in which macroscopically visible metastases were not present were used for injections. Blood and internal organs were obtained from animals which had been injected subcutaneously with tumor tissue as well as from those with tumor tissue injected into testes, spleen, and liver.

Blood was obtained from cardiac punctures conducted under sterile conditions and it was injected subcutaneously into normal rats in doses of 2-6 ml. Before they were used for injection, the organs were freed of surface membranes, washed in saline,and thoroughly minced with scissors. These preparations were injected subcutaneously into normal rats in doses of 1.5-3 ml of 5% suspensions in saline.

All the experimental animals were kept under identical conditions. Experiments lasted from February to May.

RESULTS

In the first experiment in which the tumor tissue was injected into different organs 193 rats were used. Table 1 presents the results of this experiment. When the tumor tissue was injected subcutaneously, into the lungs, tongue, or tail, there were no metastases into internal organs.

Following subcutaneous injection, the latent period of development of the tumor lasted 3-4 days and the tumor reached its biggest size at the time of death of animals on the 25th to 30th day. In section the tumors appeared as grayish-white masses with necrotic foci in the center.

Following injection into the tongue the tumors developed very intensely, filled the entire buccal cavity towards the 10th to 12th day, and caused the death of animals from starvation. Development of metastases in internal organs was not noted.

Following injection into the lungs the tumors produced a sharp dyspnea and experimental animals died after 10-12 days. Upon autopsy tumor nodes ingrown into lung tissue as well as pneumonic areas were found. Metastases were not noted in other internal organs.

When the tumor tissue was injected into the tail there was a local progressive development of the tumor tissue. Such tumors reached the size of a walnut or bigger after 20-30 days. In 4 out of 16 cases the tumors became established, increased in size, but after 10-15 days began to recede. In these rats in which tail tumors became very big, a part of the tail fell off,but the tumors continued to grow on the remaining part. These rats died after 35-40 days as a result of invasion of neighboring tissues by the tumor. Upon autopsy macroscopically visible metastases in internal organs were not seen in a single case.

Injection of tumor tissue into the testes, kidney, and liver produced metastases in the greater and the lesser omentum, lymph nodes, abdominal cavity, and peritoneum. No visible metastases were observed in organs not injected with the tumor tissue.

Injection of tumor tissue into the spleen produced a different result. In this case the development of metastases was noted in the greater and the lesser omentum, the peritoneum, abdominal lymph nodes, stomach, intestine,

Development of metastases of rat kidney carcinoma RA in the liver following injection of tumor tissue into the spleen.

diaphragm,and especially in the liver, in which metastases were not observed following injection of the tumor tissue into other organs. In the liver metastases usually developed in the medial lobe (figure).

When the tumor tissue was injected into the kidney, liver, and spleen there was development of large tumor nodes at the site of incision.

In the second experiment the animals were injected with organ suspensions and with blood of rats suffering from metastasizing and non-metastasizing forms of carcinoma. The results of this experiment are shown in Table 2. Following injections of organs from rats with metastasizing tumor, in 11 cases out of 20 there developed typical subcutaneous tumors with latent growth period of 10-12 days. In this number of positive results, 5 cases were a result of injection of liver tissue, 5 cases of spleen tissue,and 1 case of lung tissue.

Injection of kidney tissue in our experience did not result in the development of tumors. Following injection of organ suspensions from rats with non-metastasizing tumor in no case out of 32 was there development of tumor. Autopsy of rats which developed tumors as a result of injection of organ suspensions did not reveal in a single case a development of macroscopically visible metastases.

In experiments in which rats were injected with blood of animals suffering from carcinoma it was noted that tumors developed only in those cases when the blood was obtained from rats with metastasizing carcinoma and when it was injected in large volumes (4-6 ml). In rats which had developed subcutaneous tumors as a result of injection of blood containing malignant cells there were no macroscopically visible metastases in internal organs.

Thus, these data indicate that the most intensive metastasizing of kidney carcinoma RA in rats was noted following the injection of the tumor tissue into the spleen. Following injection of tumor tissue into the liver, kidney, and testes, metastases were mainly seen in the greater and the lesser omentum, peritoneum, and lymph nodes of the abdominal cavity. Injection of tumor tissue into the tail, tongue, lungs,and subcutaneously produced a local tumor process without metastases. It was possible to determine by bio-assay that viable malignant cells of kidney carcinoma RA were present in circulating blood and in some internal organs of animals suffering from this carcinoma.

In conclusion it must be said that rat kidney carcinoma RA may be used as an experimental model for the study of distribution and localization of metastases and for the study of viability and of biology of tumor cells circulating in the blood of cancerous animals.

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