SOME PECULIARITIES OF METASTASIS FORMATION BY EXPERIMENTAL TUMORS

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The study of the process of metastasis formation by malignant tumors occupies an important place in experimental oncology. Despite the extensive literature on this subject, opinions are still divided on the causes of metastasis formation by malignant tumors. Of great interest to research workers in this field is I. P. Pavlov's view of the growth of malignant tumors [5]. He believed that stimulation arising from the tumor focus, acting through a nerve reflex, can affect the nutrition of the tissues surrounding the tumor, thereby creating favorable conditions for growth of the tumor cells. I. P. Pavlov's views on the importance of trophic reflexes for the growth of tumors provided a basis for the explanation of metastasis formation and they were accordingly developed further in research by a number of workers [3, 4, 7, 8 and others].

S. I. Lebedinskaya and A. A. Solov'ev [3, 4] showed that the application of direct trauma to peripheral nerves may lead to enhanced metastasis formation and may alter the distribution of the metastases. In view of their findings, these workers consider that nervous reflex mechanisms lie at the basis of the process of metastasis formation, and determine not only the appearance of metastases but also their location. Some workers [2, 10-13] have also pointed out a definite role in metastasis formation is played by the biological peculiarities of malignant tumors. All these investigations show that the formation of metastases by malignant tumors is a complex process which depends on various factors of the external and internal environments.

The object of the present work was to study the relationship of the development of metastases to the individual peculiarities of a number of experimental tumors and to the site of introduction of the primary tumor material. We attempted to discover the strain of tumor which would be the most suitable model for the experimental study of the laws of metastasis formation.

EXPERIMENTAL METHOD

Our investigations were carried out on Ehrlich's adenocarcinoma of mice and on various tumors of rats (M-1 sarcoma and Yoshida's sarcoma, produced in rats by carcinogenic substances; Guérin's carcinoma, deriving its origin from spontaneous carcinoma of the uterus; spontaneous tumor of the mammary gland — fibroadenoma). Metastases in the experimental animals were examined macroscopically at autopsy and, in doubtful cases, microscopically.

Ehrlich's adenocarcinoma was inoculated in mice by implantation of ascitic cells in a dose of 0.2-0.5 ml of undiluted ascitic fluid in the peritoneal cavity or under the skin of the left or right flank. The rats were inoculated subcutaneously, intraperitoneally and intratesticularly with tumor tissues. Yoshida sarcoma was injected in the form of ascitic cells and a suspension of tumor fragments in physiological saline. The remaining rat tumors were inoculated by a suspension of tumor fragments only. The dose of material inoculated was the same in all cases in the experiment, namely 1 ml of a 10% suspension of tumor fragments. The experimental animals were of both sexes and were aged from 3 to 8 months.

Ehrlich's mouse adenocarcinoma. 145 mice were inoculated subcutaneously with Ehrlich's adenocarcinoma. Of these mice, 60 died from tumors between the 10th and 30th days. No metastases from Ehrlich's adenocarcinoma were found in any of the mice dying at this time. Of the 48 experimental animals dying from tumors between the 31st and 35th days, one mouse (2.08%) had metasteses in the lymphatic glands. The number of mice with metastases rose as the experimental animals died at later periods. For example, among animals dying between the 36th and 40th days metastases were observed in 9 out of 21 (44.4%), and of the 16 mice dying after the 40th day, metastases were found in 10 (62.5%). Hence, after subcutaneous inoculation of Ehrlich's adenocarcinoma metastases developed in 20 of 145 mice, or in 13.7% of cases. Metastases were found in the omentum, peritoneum, kidneys and lymphatic glands.

The highest percentage of metastases of Ehrlich's adenocarcinoma was found when the tumor was inoculated intraperitoneally. Of the 120 mice in this experiment, 115 showed the presence of metastases (95.8%). In this case the metastases were located in the omentum, mesentery, sex organs, kidneys, liver, spleen and other organs.

The experimental results show that Ehrlich's adenocarcinoma of mice metastasizes feebly after subcutaneous inoculation and much more intensively when inoculated intraperitoneally. The highest percentage of metastases from Ehrlich's adenocarcinoma after subcutaneous inoculation was observed in our experiment between the 36th and 45th days, and not at early periods as described by S. Ya. Klenitskii [1].

M-1 sarcoma of rats. In this experiment 180 rats were inoculated subcutaneously and the inoculation was successful in 127 animals. In no cases, whether the rats died at an early or late period from the tumor, were metastases found.

When M-1 sarcoma was inoculated into the testicle, metastases developed in the peritoneal cavity of 87 of the 150 rats, or in 58% of cases. The metastases were situated in the omentum, mesentery or peritoneum, on the surface of the diaphragm and in muscle tissue. Thus in contrast to Ehrlich's adenocarcinoma of mice, the M-1 sarcoma of rats does not form metastases when inoculated subcutaneously. The development of metastases from this tumor is observed in experimental animals only when it is inoculated intratesticularly and intraperitoneally.
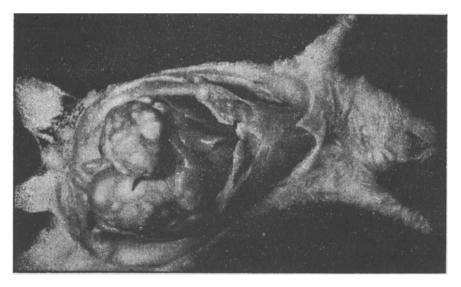
Yoshida's sarcoma of rats. This tumor was injected subcutaneously into 70 rats. Tumors developed in 62 animals (88.5%). At postmortem examination of rats dying from tumors, no metastases were seen in any animal. After intratesticular inoculation of rats with this tumor, metastases developed in the peritoneal cavity of 31 of the 40 animals. Metastasis formation from Yoshida's sarcoma has a much more intensive course when the ascitic cells of this tumor are injected intratesticularly than when a suspension of tumor fragments in physiological saline is used for the inoculation.

This is evidently due to the fact that ascitic cells of Yoshida's sarcoma are more malignant than the cells of the solid form of this tumor.

A high percentage of metastases is observed after intraperitoneal inoculation of Yoshida's sarcoma. In this experiment metastases developed in 45 of the 50 rats, or in 90% of cases. When Yoshida's sarcoma was inoculated intratesticularly and intraperitoneally, the metastases were situated mainly in the omentum, mesentery and muscle tissue, and on the surface of the diaphragm, i.e. just as in the case of metastasis formation from the M-1 sarcoma.

Guerin's sarcoma of rats. Ninety-five rats were inoculated subcutaneously with Guerin's carcinoma; 2 rats died on the 18th day without metastases. Thirty rats died from tumors between the 21st and 30th days. At postmortem examination it was found that only 2 rats had metastases in the lymphatic glands (cervical and inguinal). Of the 50 rats dying from tumors between the 31st and 35th days, metastases developed in 15 or in 30% of cases. Of 8 rats dying between the 36th and 40th days, 7 showed the presence of metastases (87.5%). Finally, all 5 rats dying at later periods were affected with metastases (100%).

Metastases from Guerin's carcinoma following subcutaneous inoculation were situated at later stages not only in the lymphatic glands but also in the peritoneal cavity, thyroid gland, adrenals, omentum, muscle tissue and mesentery. However the favorite site for the formation of metastases from Guerin's carcinoma after subcutaneous inoculation is the lymphatic glands. After intratesticular inoculation of rats with Guerin's carcinoma, the course of development of the tumor is somewhat different from that of the M-1 sarcoma or Yoshida's sarcoma

Development of a fibroadenoma of the mammary gland after its autotrans-
plantation into the peritoneal cavity of an experimental rat. On the 124th
day of the experiment massive tumor nodes are seen in the omentum, on the
surface of the diaphragam and in the mesentery.

inoculated into the same organ. In our experiment the development of Guérin's carcinoma in the testicle was accompanied by relatively feeble matastasis formation, which in some rats was confined to the tissues close to the testicle. Of the 50 rats used in this experiment, metastases from Guérin's carcinoma developed in the peritoneum of 17 animals, or 34% of cases. The first metastases were formed in the omentum, mesentery and peritoneum. In some rats no metastases were observed in the peritoneal cavity during development of a Guérin's carcinoma in the testicle. In this case the tumors grew as a continuous conglomerate mass, quite separable from the adjacent tissues. This fact is all the more interesting because the testicle, according to the reports of many workers, is the organ from which metastases are formed by tumors of even a low degree of malignancy.

When inoculated intrapertoneally, Guérin's carcinoma metastasized in 19 of the 30 rats, or in 63.3% of cases. In some rats the development of Guérin's carcinoma in the peritoneal cavity was accompanied by ascites formation. The ascitic fluid contained blood corpuscles and the characteristic malignant cells of the ascitic form of the tumor. Subcutaneous inoculation of rats with these cells caused the development of typical carcinomas with a relatively short latent period of 2-3 days, followed by death of the rats on the 20th-25th day.

Thus in relation to its ability to form metastases, Guérin's carcinoma of rats differs essentially from the M 1 sarcoma and Yoshida's sarcoma of rats. After subcutaneous inoculation, Guérin's carcinoma metastasizes in 30.5% of cases, but the sarcomas do not form metastases in this case. Furthermore Guérin's carcinoma metastasizes directly in the internal organs (lymphatic glands, thyroid gland); according to our findings, these organs are not affected by metastases from M-1 and Yoshida's sarcomas.

Spontaneous mammary gland tumor (fibroadenoma) of rats. Fibroadenoma of the mammary gland is known to be a malignant tumor and consequently not to form metastases. The animals die from general exhaustion because of the intensive growth of this tumor.

In our experiment between 1953 and 1958 we observed 6 cases of fibroadenoma of the mammary gland in rats, and in no case were metastases formed from this tumor. Homotransplantation of a fibroadenoma intratesticularly in 9 rats and intraperitoneally in 26 rats was unsuccessful in all cases, in spite of the fact that the rats inoculated were genetically similar to the donors. In our experiments we therefore employed the generally known method of autotransplantation, described in detail by N. N. Petrov [6]. Autotransplantation of the fibroadenoma was carried out subcutaneously and intraperitoneally. For intraperitoneal inoculation 6 rats affected by tumors were used; in 4 of these animals massive tumor nodes weighing from 90 to 120 g developed in the peritoneal cavity after $3\frac{1}{2}$ to 4 months. Tumor nodes were located in the omentum and mesentery and on the surface of the diaphragm.

This fact suggests that a fibroadenoma of the mammary gland may grow and develop not only at the site of its original appearance but in other parts of the body also. Evidently the peritoneal cavity is the most favorable environment for the growth of benign as well as malignant tumors.

It may be concluded from the results obtained that Guerin's carcinoma of rats is the most suitable for studying the process of metastasis formation by malignant tumors, for it forms metastases when inoculated subcutaneously, intratesticularly and intraperitoneally. Ehrlich's adenocarcinoma of mice metastasizes less intensively than Guerin's carcinoma when inoculated subcutaneously, and so is less suitable for studying this process. The M-1 sarcoma and Yoshida's sarcoma of rats were least suitable of all for the experimental study of the process of metastasis formation.

The metastasization of malignant tumors is a complex process in which, besides other factors, a large part is played by the situation of the primary tumor and its biological peculiarities. A benign tumor, such as a fibroadenoma of the mammary gland, may also grow when cells from it are introduced into the peritoneal cavity of the animal affected by the primary tumor. Under these circumstances, however, the internal organs (liver, kidneys, spleen, lungs, etc) are not affected by metastases.

SUMMARY

Metastasis of transplantable malignant tumors is a complicated process in which the localization of the primary tumor and its biological peculiarities together with other factors play an important role. Guerin rat's carcinoma, which metastasizes into internal organs both in subcutaneous inoculation and in its graft into the testicle or into the abdominal cavity, was found to be the most suitable for the study of the laws of metastasis of malignant tumors. Rat's M-1 sarcoma and Yoshida's sarcoma are the least suitable for the study of this process.

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