TRANSPLANTATION OF EXPERIMENTAL OVARIAN TUMORS AND THEIR SENSITIVITY TO CARCINOSTATICS

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The transplantation of experimental ovarian tumors induced by chemical carcinogenetic materials to rats and their sensitivity to carcinostatics were discussed.

Among the rats of the same series to which tumors were tried to transplant subcutaneously, sarcoma was successfully transplanted in 2 lineages; one is in the 52nd generation and another is in the 44th generation at present.

The tumors could be transplanted to both male rats and female rats of the same series, but the transplantation rate was different in early generations. Tumor formation was seen not only by transplanting tumor cells subcutaneously but by injecting them intraperitoneally.

The sensitivities of the transplanted tumors to various carcinostatics were tested and Mitomycin C was found to have a surviving effect.

INTRODUCTION

It has been performed since long before to induce human malignant tumor experimentally in animal and to study its etiology and pathogenesis to reveal the relation between the induced tumor and human tumor.

Many reports are available in the obstetric and gynecological field; particularly, as to experimental uterine cancer, the technique to induce the cancer has been improved and the relation between the induced tumor and human tumor is revealed (Taki and Iijima, 1963; Iijima and Taki, 1964).

On the other hand, there are established experimental methods to induce some of human ovarian tumors relating to hormonogenesis such as granulosatheca cell tumor, and the pathogenetic processes are widely discussed, but no method seems to be established as to other type of ovarian tumors.

Since 1973, the authors have studied the method to induce experimental ovarian tumors on rats of Wistar series and succeeded in inducing adenocarcinoma efficiently by the clipping method which was developed in our laboratory. The method and the tumor producing process were reported (Kato, Yakushiji, et al. 1973, 1974, 1975).

The present report describes the transplantation of experimental ovarian tumors induced by the clipping method and the sensitivity to carcinostatics in the rats in which the tumor was successfully transplanted to established strains.

METHODS

The induced ovarian tumors were re-
moved aseptically and some part was observed histologically and some was transplanted.

The technique of the transplantation was as follows: a coat of tumor and its necrotic part completely removed, and the tumor was precisely cut; a penicillin solution of 2000-3000 IU was dripped, and 1 ml of the obtained solution was injected subcutaneously on the back of female rats of a series by a transplantation needle. The solution was partially injected intraperitoneally.

RESULTS

1. Transplantation in the first generation

Among the experimental ovarian tumors induced, sarcoma was transplanted to 6 rats, adenocarcinoma to 18 rats, and granulosa cell tumor to 1 rat, and the subcutaneous transplantation to the first generation was succeeded in 3 rats with sarcoma and 1 rat with adenocarcinoma. Sarcoma was transplanted intraperitoneally to 3 rats and succeeded in 1 rat.

2. Serial transplantation

Subcutaneous serial transplantation of sarcoma was succeeded in 2 out of 3 rats in which first transplantation was successful. Fig. 1 a), b) shows the transplantation rate in the 2 lineages and the processes to the death by the tumor. The transplantation rate was kept at 90-100% after the 12th generation of No. 24 rats and the 20th generation of No. 142 rats. The average period to the death by the tumor was about 30 days in No. 24 and about 40 days in No. 142. Fig. 2 and Fig. 3 show the macroscopic findings and the histological picture of the transplanted tumor in the 27th generation of No. 24.

3. Host conditions and transplantation

The transplantation rate in No. 24 rats was evaluated under the two host conditions: 1) castrated female rats and 2) male rats.

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Fig. 1-a Transplantation rate.

Fig. 1-b Mean survival period.

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Fig. 1.
**Fig. 2.** Macroscopic finding of the transplanted tumor.

**Fig. 3.** Macroscopic finding of the transplanted tumor.

**TABLE 1**

*Host conditions and transplantation*

<table>
<thead>
<tr>
<th>Host conditions and transplantation</th>
<th>a) Transplantation rate to castrated female rats (%)</th>
<th>b) Transplantation rate to male rats (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>generation</td>
<td>8 13 19</td>
<td>22 23</td>
</tr>
<tr>
<td>experimental group</td>
<td>40 60 70</td>
<td>70 80</td>
</tr>
<tr>
<td>control group</td>
<td>90 100 100</td>
<td>100 90</td>
</tr>
</tbody>
</table>

**Fig. 4.** Changes in the vaginal smear of the host.
Table 1 shows the transplantation rate in each generation of No. 24 rats. The rate in the 8th generation of the castrated female rats was lower than that in the control of non-castrated female rats, but tended to increase up to 60% and 70% in the 13th and the 19th generations.

The rate in the male rats of the 22nd and the 23rd generations was 70% and 80% respectively, a little lower than that in the control and higher than that in the castrated female rats in earlier generations.

4. Changes in the vaginal smear of the host

The change of the estrous cycle in the tumor transplanted female rats was investigated based on the vaginal smear collected at 10:00 a.m. every day. The results are shown in Fig. 4. The estrus revolved periodically, though a little irregularly, in the non-transplantation group, whereas in the tumor-transplantation group it revolved irregularly to disturb the cycle.

5. Sensitivity of the transplanted tumors to carcinostatics

The sensitivity of sarcoma strains of No. 24 rats to various carcinostatics was tested.

Table 2 show the sort, dose and route of the given carcinostatics, and Fig. 5 shows their effects on the surviving term.

Among 3 carcinostatics given, Mitomycin C prolonged most the surviving term. 5-FU and Endoxan did not show any prominent effects.

DISCUSSION

The study of the implantable tumor has a long history. In 1889, Hanau transplanted to albino rats cancroid which was formed in a vagina of a albino rat and showed lymphnode metastasis. He autopsied the cancroid transplanted rats which died 6 weeks after the transplantation and found tumor cells in the abdominal cavity. Loeb (1901, 1902) and Jensen (1903) succeeded in serial transplantation of cancer to mice and rats, and the experimental carcinology started.

Recently, some cases of successful transplantation of experimental tumors were reported and in Japan a list of implantable tumors was published by Japanese Society of Cancer in 1978.

On the other hand, it has been long tried to induce ovarian tumor experimentally; there are reported some methods to induce the tumor by X ray irradiation (Furth and Furth, 1936; Butterworth, 1973), ovarian transplantation to spleen (Biskind and Biskind, 1944, 1948) and chemical carcinogenetic substances (Engelbreth-Holm, 1939).

The relation between granulosa cell tumor induced by ovarian transplantation to spleen and hormones has been revealed, but not so many reports are available on adenocarcinoma which most frequently appears among ovarian tumors. The authors have succeeded to induce adenocarcinoma efficiently by imbedding and fixing chemical carcinogenetic substances into the ovarian parenchyma.

There are few reports dealing with the transplantation of experimental tumor of ovarium-origin, and no report available on the cases in which strains are established. In our experiment with sarcoma, strains were established in 2 cases.

Some investigators studied hormon dependency of experimental tumors selecting host conditions and other factors and showed their relationship: Bonser (1944) with testicular interstitial cell tumors, Gardner (1945), and Jull (1954).
Fig. 5. Sensitivity of the transplanted tumors to carcinostatics.

TABLE 2

<table>
<thead>
<tr>
<th>Carcinostatics</th>
<th>dose and administration</th>
<th>Total dose</th>
<th>No. of rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitomycin</td>
<td>1000 mcg/kg → 1 × 4 days × 7 (subcut)</td>
<td>7000 mcg/kg</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>500 mcg/kg → 1 × 4 days × 7 (subcut)</td>
<td>3500 mcg/kg</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>200 mcg/kg → 1 × 4 days × 7 (subcut)</td>
<td>1400 mcg/kg</td>
<td>5</td>
</tr>
<tr>
<td>Endoxan</td>
<td>20 mg/kg → 1 × 4 days × 7 (subcut)</td>
<td>140 mg/kg</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>10 mg/kg → 1 × 4 days × 7 (subcut)</td>
<td>70 mg/kg</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>5 mg/kg → 1 × 4 days × 7 (subcut)</td>
<td>35 mg/kg</td>
<td>5</td>
</tr>
<tr>
<td>5-Fu</td>
<td>50 mg/kg → 1 × 4 days × 7 (subcut)</td>
<td>350 mg/kg</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>25 mg/kg → 1 × 4 days × 7 (subcut)</td>
<td>175 mg/kg</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>10 mg/kg → 1 × 4 days × 7 (subcut)</td>
<td>70 mg/kg</td>
<td>5</td>
</tr>
</tbody>
</table>
The authors performed the experiments for the same purpose varying host conditions and obtained a possible transplantation rate which was lower than that in the control using castrated female rats of 8th generation. However, it tended to become gradually higher in the rats from the 13th to the 19th generations. In the male rats of the 22nd and the 23rd generations it was lower than that in the control. These results appear to indicate that the transplantation rate depends on the host condition. But, further investigation would be required as to the hormone dependency.

The vaginal smear changed in the tumor-transplanted animals. This may be rationally explained not by the relation between the transplanted tumor and hormones but by secondary changes following the enlargement of the tumor.

The fundamental theory of chemotherapy was founded by Ehrlich in an early stage of the 20th century, and since then Huggins and other investigators demonstrated a possibility of castration and estrogen treatment for prostatic carcinoma and then started modern chemotherapy for carcinoma. In these years many chemical agents have been tried empirically and screening of their carcinostatic effects to which Stock & Sujiura's animal screening greatly contributed has attracted investigator's attention. There are many other in vivo and invitro screening methods such as Karnofsky's method based on the growth depression on chick embryo using eggs. More recently Rygaard et al. (1969) reported that human carcinoma can be easily transplanted to nude mice, and Povlsm et al. (1975) transplanted human carcinoma to nude mice and tested the sensitivity to carcinostatics. Thus, the investigation in this field is extensively being carried out, but it is pointed out that the tumors transplanted to nude mice are of no practical use because their growth is too slow, and that the subcutaneous transplantation to nude mice makes it necessary to evaluate the results taking into consideration of the distribution of carcinostatics in organs.

Thus there remains many problems in the application to human beings of the sensitivity tests on the transplanted tumor, but our results indicate that Mitomycin C is expected to have the best surviving effect.

REFERENCES


