Tumor Induction In Transplanted Mammary Glands in Rats

THOMAS L. DAO, YOSHIMORI TANAKA, and DANIEL GAWLAK, Department of Breast Surgery, Roswell Park Memorial Institute, Department of Health, State of New York, Buffalo, New York

SUMMARY

Mammary glands were successfully transplanted from randombred Sprague-Dawley rats to recipients, irrespective of whether the transplants were autografts or homografts. Homologous transplantation of mammary glands was successful in both littersmates and nonlittermates. Surviving grafts had the histological appearance of normal mammary glands and functioned like the normal mammary glands of the recipients. The grafts showed extensive lobular-alveolar growth, secreted milk when the graft-bearing rats were pregnant, and atrophied, as the mammary glands of the recipients did, when their ovaries were removed. When female rats were fed a single dose of 7,12-dimethylbenz[α]-anthracene (DMBA) and their mammary glands were subsequently transplanted into female recipients, mammary cancer developed in the mammary grafts in the hosts. Mammary cancer developed in a gland transplanted to a recipient as soon as 4 hours after the donor was fed a single dose of DMBA. The incidence of tumors was significantly lower in mammary grafts from male donors than from female donors. Mammary tumors developing in such grafts are hormone-dependent, since they regress after excision of the ovaries.—J Nat Cancer Inst 32: 1259–1275, 1964.

CARCINOGENESIS IN transplanted mammary glands has been studied previously in mice. Shimkin et al. (1) reported transplantation of mammary tissue in 67 mice, with only 1 tumor appearing at the site of transplantation. Prehn (2) reported transplantation of mammary glands by grafting the skin and attached glands on the recipient animal. The transplantability of the grafts and the relationships between hormones and growth of the grafts were not described by these investigators. Faulkin and DeOme (3) reported the transplantation of hyperplastic nodules and mammary glands and the later development of mammary tumors from the transplanted nodules. Recently Hoshino (4) described the transplantability and growth potentiality of mammary glands in mice. There are no previous reports in the literature, however, of studies of the transplantation of mammary glands in rats.

1 Received October 24, 1963.
2 Supported by grant CA-04632 from the National Cancer Institute, National Institutes of Health, Public Health Service.
The present paper reports the successful transplantation of mammary glands to male and female rats from both littermates and nonlittermates. These grafts retained all the characteristics of hormone dependency of normal mammary glands. We also experimented to ascertain whether mammary cancer will develop in a mammary graft when a chemical carcinogen is given to the donor rat before transplantation, and to determine to what extent the mammary cancer developing in the mammary graft in the recipient rat is hormone-dependent.

**MATERIALS AND METHODS**

Rats of both sexes, supplied by the Holzman Company, Madison, Wisconsin, were originally derived from the Sprague-Dawley strain and have been random bred by the Holzman Company since 1946. At the time of mammary transplantation, the animals were 60 to 70 days old and weighed 165 to 195 g. They were fed a commercial ration (Rockland diet) and were given water *ad libitum*.

The transplantation technique involved was simple. Under ether anesthesia, the abdomino-inguinal mammary gland was removed from the donor and immediately transplanted in the recipient. After the development of a skin flap of appropriate size on the dorsum, an area normally free from mammary tissue, the mammary gland to be transplanted was spread out flat and fixed to the fascia with 8 to 10 silk sutures. The skin incision was closed with clips. During the surgical procedure the gland was kept constantly moist with sterile isotonic saline.

The rats studied comprised the following experimental groups: (a) 10 female rats whose mammary glands were autotransplanted; (b) 15 females receiving mammary gland grafts from littermate females; (c) 15 females given grafts from littermate males; (d) 15 males receiving grafts from littermate females; 3 groups (e, f, g) exactly paralleling groups (b), (c), and (d), except that the mammary glands were from nonlittermate donors; (h) 5 females bearing mammary transplants, mated and becoming pregnant 10 days after transplantation; (i) 5 females with mammary grafts, ovariectomized 20 days after transplantation; and (j) 5 males bearing mammary grafts, orchiectomized 20 days after transplantation.

Rats in group (a) were killed 10 days after autologous transplantation. In groups (b) to (g), 5 in each group were killed 10, 20, and 30 days after transplantation. In group (h), the rats were killed during the last trimester of pregnancy. The mammary graft and 1 inguinal mammary gland were removed from each animal at autopsy. The animals in groups (i) and (j) were killed a month after castration. Both the graft and the mammary gland were studied histologically or by the wholemount technique.

The basic experimental procedure in the study of carcinogenesis in mammary gland grafts was as follows: Every donor rat was fed a single
TUMOR INDUCTION IN RAT MAMMARY GLANDS

A dose of 20 mg 7,12-dimethylbenz[a]anthracene (DMBA) in 1 ml of olive oil. In each of 4 subgroups of 10 rats each, mammary gland transplantation was then carried out at one of the following times after the carcinogen was fed: 4 hours, 24 hours, 5 days, and 10 days. The 2 abdomino-inguinal mammary glands were carefully dissected from each donor, and 1 gland was immediately transplanted into each of 2 littermates by the techniques described.

There were 4 groups of experimental animals. Donors and recipients in the 4 groups were as follows: 1) female donors, 30 to 35 days old, and female recipients the same age; 2) male donors, 30 to 35 days old, and female recipients the same age; 3) female donors, 55 to 60 days old, and female recipients the same age; and 4) male donors, 55 to 60 days old, and female recipients the same age.

The recipients were examined regularly for the development of mammary cancer in the graft. At the end of 6 months, all rats bearing mammary grafts were killed. Each graft was examined grossly and dissected carefully for histological sections and wholemount studies.

Bilateral ovariectomy was done in 5 recipients bearing mammary gland grafts that had developed tumors. A biopsy specimen was taken from each tumor before ovariectomy. The growth rate of the mammary cancer in the graft was measured at regular intervals both before and after ovariectomy. When the mammary cancer in the graft regressed after ovariectomy, a pair of ovaries was implanted subcutaneously. The growth rate of the mammary cancer in the graft was again measured regularly after ovarian grafting.

RESULTS

Gross and Microscopic Appearance of Surviving Mammary Grafts

The surviving mammary glands were well vascularized and normal appearing, with no evidence of necrosis (fig. 1). The fat pad, pearl-white at the time of transplantation, became yellowish, suggestive of degenerative changes, but the histological appearance of fat cells was normal.

Viable mammary gland grafts looked normal histologically like those of the recipients' own mammary glands (figs. 2 and 3). This normal appearance was unchanged regardless of whether the grafts were examined 10, 30, or 180 days after transplantation.

The only histological difference between a viable mammary graft and a mammary gland of the host was the appearance of the lymph node. The well-encapsulated lymph node of the normal mammary gland was no longer identifiable in a graft. Instead, an area with a large accumulation of lymphocytes occurred without any definite capsule in the periphery. Adjacent to it were areas of stroma with lymphocytic infiltration. No degenerative changes were seen, however.
Autologous Transplantation of Mammary Glands

In 10 female rats, the mammary gland transplanted autologously from the right abdomino-inguinal region to the back survived in every case. Histologically, these viable grafts looked essentially the same as the graft of the left abdomino-inguinal mammary gland, which served as a control.

Transplantation of Mammary Glands in Littermate Rats

At least 3 of 5 grafts took successfully (table 1). The mammary glands from male donors were successfully transplanted into female recipients. The alveolar lobules in an adult, male rat mammary gland were not only maintained but also grew when the gland was transplanted into a female recipient. The presence of alveolar lobules in adult, male rat mammary glands is a normal phenomenon and has been described previously (5, 6). However, grafts from a female donor growing in male recipients are often thinner and their ductal growth is less abundant.

**Table 1.—Homologous transplantation of mammary glands in rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of viable grafts</th>
<th>Total No. of rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 days after transplantation</td>
<td>20 days after transplantation</td>
</tr>
<tr>
<td>Female→female (littermates)</td>
<td>4/5</td>
<td>4/5</td>
</tr>
<tr>
<td>Male→female (littermates)</td>
<td>4/5</td>
<td>3/5</td>
</tr>
<tr>
<td>Female→male (littermates)</td>
<td>3/5</td>
<td>4/5</td>
</tr>
<tr>
<td>Female→female (nonlittermates)</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>Male→female (nonlittermates)</td>
<td>4/5</td>
<td>5/5</td>
</tr>
<tr>
<td>Female→male (nonlittermates)</td>
<td>4/5</td>
<td>4/5</td>
</tr>
</tbody>
</table>

Transplantation of Mammary Glands in Nonlittermates

The results listed in table 1 demonstrate that mammary glands can be successfully transplanted into nonlittermate male and female rats. The histological appearance of viable mammary grafts was the same as that of a normal mammary gland of the host.

Effect of Pregnancy and Oophorectomy on Growth of Mammary Grafts

Five rats bearing mammary gland grafts from littermates were mated and became pregnant 10 days after transplantation. The mammary gland grafts showed extensive lobular-alveolar growth and secreted milk, as did the mammary glands of the hosts (fig. 4). In some pregnant rats...
bearing mammary grafts, the transplants could be easily palpated through the skin during the last trimester of pregnancy.

The mammary gland grafts in 4 of 5 female rats greatly atrophied 4 weeks after bilateral ovariectomy. The mammary gland graft in one rat, however, did not atrophy appreciably.

When a male animal bearing a surviving female mammary gland graft was castrated, the lobules in the female mammary gland graft disappeared. This phenomenon was likewise observed in the recipient's own mammary glands.

Development of Mammary Tumors in Surviving Grafts in Recipient Rats

When mammary glands from female donors were transplanted into females, mammary adenocarcinomas could be palpated in the graft as early as 60 days after transplantation. (Since stitch abscesses along the edges of grafts are not uncommon, diagnosis of mammary adenocarcinomas was made only by biopsy or autopsy.) Histologically, the tumors in the grafts resembled those induced in mammary glands by oral administration of DMBA (fig. 5). A tumor in a graft can attain enormous size (fig. 6) or grow slowly as a small tumor which can be identified only in a wholemount preparation or in histological sections (figs. 7 and 8). Most tumors in grafts, however, rarely grow larger than 2 cm in diameter. Central necrosis was frequently observed in these tumors. Among the mammary tumors in 45 rats, 41 were adenocarcinomas, 1 was a sarcoma, and 3 were fibroadenomas (figs. 9 and 10). Mammary hyperplasia was observed in 7 grafts (fig. 11). Whether these hyperplastic lesions were early neoplastic changes in the mammary graft could not be ascertained.

The incidence of tumors is shown in table 2. The incidence of mammary cancer is significantly lower in grafts from male donors than from female donors. The relationship of the time interval between feeding of the carcinogen and grafting to the incidence of mammary tumors in the grafts is not evident and further investigation of this factor is needed. However, mammary glands transplanted from male donors to female recipients shortly after carcinogen feeding failed to develop as many mammary tumors as glands taken at the same time from female donors.

Under the conditions of the present study, the age of the donor did not seem to influence the incidence of cancer in mammary grafts. From our observations, no recipient of a mammary graft developed a tumor in one of her own mammary glands.

Effect of Ovariectomy on Mammary Adenocarcinoma in the Graft

In all 5 instances of ovariectomy after the development of palpable mammary cancer in mammary grafts, the tumors decreased in size or became nonpalpable. Histologically, the columnar epithelial cells in the tumors in the ovariectomized rats were now replaced with a single layer of flattened cuboidal cells.
TABLE 2.—Transplantation of mammary glands and development of tumors in mammary grafts in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time between</th>
<th>Number of rats</th>
<th>Number of rats with tumors</th>
<th>Number of rats in surviving grafts</th>
<th>Number of rats with tumors in surviving grafts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>carcino-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>gen feeding</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>and grafting</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>4 hours</td>
<td>10</td>
<td>6</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>24 hours</td>
<td>10</td>
<td>8</td>
<td>3†</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>5 days</td>
<td>10</td>
<td>9</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>10 days</td>
<td>10</td>
<td>9</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Males</td>
<td>4 hours</td>
<td>8</td>
<td>5</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>24 hours</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>5 days</td>
<td>10</td>
<td>8</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>10 days</td>
<td>9</td>
<td>7</td>
<td>1</td>
<td>8</td>
</tr>
</tbody>
</table>

*The original number of rats in each group was 10. A lesser number in this column indicates exclusion due to postoperative death.
†One tumor was a sarcoma, 1 a fibroadenoma, and 1 an adenocarcinoma.
‡Both were fibroadenomas.

When a pair of ovaries was grafted into an ovariecotomized rat, the regressed mammary tumor in the mammary graft increased in size again in 3 of 5 recipients.

DISCUSSION

The present study shows that mammary glands can be successfully transplanted into both littermate and nonlittermate rats. The successful transplantation of mammary glands in randombred rats raises the question whether these animals have attained a great degree of inbreeding. Is the transplantation thus isologous rather than homologous? Although this is certainly a reasonable possibility, experiments with cross-skin grafts must be done to ascertain the degree of inbreeding. Such experiments are now being carried out in this laboratory.

The viability of the grafts was assessed by both histological appearance and functional capacity to grow and secrete under hormonal stimulation. The female grafts in females showed extensive lobular-alveolar growth and secreted milk when the graft-bearing rats were pregnant. When rats were ovariecotomized 20 days after transplantation of mammary glands, the grafts atrophied, just as did the mammary glands of the recipients. These results demonstrate that mammary gland grafts respond to hormonal regulation in the same general way as the recipients' own mammary glands.

The ductal-alveolar growth of the female mammary graft was maintained in male recipients. Orchiectomy caused lobular growth to decrease or disappear. The loss of lobular-alveolar growth in the mammary graft after orchiectomy suggests that hormones from testes are as effective as...
hormones from ovaries in stimulating the growth of mammary glands.

The fact that mammary glands of male rats can be transplanted into female recipients indicates that male mammary glands can develop fully in a suitable host.

Interestingly, mammary cancer can develop in a mammary gland transplanted from a donor given DMBA before the transplantation. Mammary tumors developing in the graft are hormone-dependent, since they regress after excision of the ovaries.

There are 2 undisputed facts about transplantation of mammary tissues: 1) The tissues are severed from their normal vascular connections, and 2) it takes time to establish a graft. Evidently, survival of a graft is a requisite to the development of a mammary tumor in the graft. Mammary cancer cannot grow in a degenerated mammary gland graft but can develop in a gland transplanted to a recipient as early as 4 hours after the donor is fed a single dose of DMBA. It is not known whether neoplastic transformation of the mammary cells occurs in the donor immediately after the cells are affected by the carcinogen, or whether the recipient has the major role in the eventual development of tumor, but answers to these questions are being sought in our subsequent investigations.

The sex of the donor may be important in the initiation of carcinogenesis in mammary gland grafts, since the incidence of tumors is significantly lower in mammary glands from male donors than in mammary glands from female donors.

Although the present study suggests that, as the time between feeding of the carcinogen and grafting of the gland increases, the incidence of tumors in grafts decreases, the implications of this observation have yet to be fully elucidated. The rate of clearance of carcinogenic hydrocarbon in the mammary gland graft, after oral administration of the carcinogen to the donor, is being investigated in our laboratory.

REFERENCES


PLATE 153

FIGURE 1.—Well-vascularized and normal-appearing mammary gland graft in female recipient 20 days after transplantation.

FIGURE 2.—Normal histological appearance of mammary gland graft removed from female recipient 20 days after transplantation. × 50
PLATE 154

FIGURE 3.—Histological appearance of recipient’s own mammary gland (same rat as shown in fig. 2). × 50

FIGURE 4.—Extensive lobular-alveolar growth and milk secretion in mammary gland graft in pregnant rat. × 50
Figure 5.—Histological appearance of adenocarcinoma developing in mammary graft 60 days after transplantation.  × 200

Figure 6.—Mammary tumor developing in mammary graft has reached an enormous size.

Figure 7.—Wholemount shows 3 small tumors in mammary gland graft. Tumors were found at time of autopsy 6 months after transplantation. Note also normal appearance of mammary gland graft.  × 10
PLATE 156

FIGURE 8.—Microscopic appearance of adenocarcinoma in mammary graft. On gross examination at autopsy, tumor appeared as small, white nodule in graft. × 10

FIGURE 9.—Sarcoma developing in mammary graft from female donor fed single dose of DMBA 24 hours before transplantation. × 100
PLATE 157

**Figure 10.**—Histological appearance of fibroadenoma developing in mammary graft from male donor fed single dose of DMBA 10 days before transplantation. Tumor was not palpable. × 7

**Figure 11.**—Hyperplasia of mammary epithelium in mammary graft from female donor fed single dose of DMBA 24 hours before transplantation. × 5