Effect of Pharmacological Doses of Estrogen on Ovary-independent Rat Mammary Carcinoma Containing Estrogen and Progesterone Receptors

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ABSTRACT

Mammary carcinoma induced in male Sprague-Dawley rats by multiple intragastric intubations of 7,12-dimethylbenz(a)anthracene showed ovary-independent growth but contained estrogen receptors (ER) and progesterone receptors (Yoshida, Yoshida, Fukunishi, Sato, Okamoto, and Matsumoto, Cancer Res., 42: 2434–2439, 1982). Transplantable carcinoma (MT6) was obtained from dimethylbenz(a)anthracene-induced mammary carcinoma and then maintained in male rats. MT6 tumors with ER grew equally well in males, females, gonadectomized males, and males given injections of bromocriptine (1 mg/day) or lisuride hydrogen maleate (50 μg/day), and gonadactomized males receiving smaller doses of 17β-estradiol (1 to 100 μg/2 days). However, the growth of MT6 was inhibited markedly by injection of a very large amount of 17β-estradiol (1 mg/2 days). Although grafted MT6 tumors were ductal carcinoma with cibiform pattern with ER, tumors recurring after injection with 1 mg 17β-estradiol were found to be spindle cell carcinoma without ER, which could grow equally well in recipients treated with or without 1 mg 17β-estradiol. These observations suggest that the growth of ovary-independent MT6 tumors with ER and progesterone receptors is inhibited only by pharmacological doses of estrogens and that the loss of growth-inhibiting effect of pharmacological doses of estrogen on MT6 tumors occurs during high-dose estrogen treatment.

INTRODUCTION

We reported that mammary carcinoma induced in male Sprague-Dawley rats by multiple i.g.3 intubations of DMBA (MM) was ovary independent, since original and transplanted MM grew equally well in male and female rats with or without gonadectomy (21). An ovary-independent transplantable tumor (MT6) was obtained by s.c. implantation of explant from MM in male rat and then maintained in male rats. These ovary-independent MM and MT6, like ovary-dependent mammary carcinomas induced by DMBA in female rats, contained ER and estrogen-dependent PGR (21). Recently, we obtained preliminary information that the growth of MT6 was markedly inhibited by pharmacological doses of 17β-estradiol. It is now generally appreciated that 170 to 80% of human breast carcinomas with positive ER and PGR are likely to regress following endocrine therapy such as estrogen removal and addition of pharmacological doses of estrogens, whereas those tumors lacking ER and PGR usually fail to respond (12, 15), and that endocrine therapy with pharmacological doses of estrogens is effective mainly in older postmenopausal patients with advanced breast cancer containing receptors (19). Since the mechanism of tumor regression by pharmacological doses of estrogens in receptor-positive breast cancer is still unknown, effects of large amounts of 17β-estradiol on ovary-independent MT6 with positive ER and PGR were investigated in the present study.

MATERIALS AND METHODS

Animals and Tumors. All animals were inbred Sprague-Dawley rats maintained in a filtered-air laminar flow enclosure and were given commercial rations and tap water ad libitum. Seed tumors for transplantations were obtained from the 14th to 16th generations of MT6, which had been obtained by s.c. implantation of explant from ovary-independent MM in male rat and then maintained in male rats. In each experiment, intact or gonadectomized female and male rats were grafted with the same seed tumors in order to ensure biological characteristics of the tumors. Seed tumors were obtained within 60 days after transplantation of MT6 in male rats.

Injection. 17β-Estradiol (1 to 1000 μg) dissolved in 0.05 ml of ethanol/sesame oil (1:4) was injected i.m. at 2-day intervals. CB-154 was dissolved in 70% ethanol and diluted with 0.85% NaCl solution to a final concentration of 15% ethanol, and 1 mg CB-154 dissolved in 0.05 ml was injected daily. Fifty μg of LHMM were dissolved in 0.05 ml of 0.85% NaCl solution.

Transplantation. Tissue fragments of approximately 100 mg obtained from MT6 tumors were transplanted by s.c. injections (No. 13 tricorn) in upper milk lines of recipients at 50 days of age. Recipient groups were as follows: Group 1, intact males; Group 2, males gonadectomy (a) on the day of or (b) on the 60th day after transplantation; Group 3, intact males given daily injections of 1.0 mg CB-154, starting from the day of transplantation; Group 4, intact males given daily injections of 50 μg of LHMM starting from the day of transplantation; Group 5, intact males given injections of 1 mg 17β-estradiol at 2-day intervals starting (a) from the day of or (b) from the 60th day after transplantation; Group 6, males gonadectomy on the day of transplantation and injected with 10, 100, or 1000 μg 17β-estradiol at 2-day intervals starting from the day of transplantation. Eight to 10 rats per group were used.

Retransplantation. Eight tumors were obtained from orchietomized rats (Group 2a) 70 to 90 days after transplantation of MT6, and 8 tumors were obtained from 17β-estradiol-injected (1 mg/2 days) orchietomized rats 130 to 170 days after the transplantation of MT6. Seed tumors from these 16 tumors were retransplanted in intact or gonadectomy males given injections with or without 17β-estradiol (1 mg/2 days), starting from the day of retransplantation, in order to examine biological characteristics. Eight rats per group were used.

Determination of Tumor Growth. The increase in size of transplanted and retransplanted tumors was monitored by vernier caliper measurements of the s.c. mass, and the product of the length and width was used as an index of tumor size.

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2 To whom requests for reprints should be addressed.
3 The abbreviations used are: i.g., intragastric; DMBA, 7,12-dimethylbenz(a)anthracene; MM, DMBA-induced mammary carcinoma in the male Sprague-Dawley rat; MT6, transplantable mammary carcinoma established from MM; ER, estrogen receptors; PGR, progesterone receptors; CB-154, 2-bromo-α-ergocryptine mesylate (bromocriptine); LHMM, lisuride hydrogen maleate.

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Histology. Tumors were fixed in 10% formalin, and the widest-cut surfaces were sectioned, stained with hematoxylin and eosin, and examined histopathologically.

Assay for Cytosol ER. ER were measured in cytosols from the retransplanted tumors grown in intact male rats without 17β-estradiol injection. The assay method for ER in tumor cytosol was described previously (15). In short, ER were determined by the dextran-coated charcoal assay using 17β[3H]estradiol (111 Ci/mmol; New England Nuclear) as radioactive steroid. The number of binding sites and the dissociation constant (Kd) were calculated according to the procedure of Scatchard (18). At most, tumors were stored for 5 weeks at -80° until assayed.

RESULTS

Growth of MT6 Tumors in Various Recipients. The growth of MT6 in various recipients is shown in Charts 1 and 2. MT6 tumors grew equally well in males, castrated males, and males treated with CB-154 or LHM. However, the growth of MT6 tumors was inhibited markedly by injection of 1 mg 17β-estradiol starting from the day of or from the 60th day after transplantation. The growth of ovary-independent MT6 tumors was inhibited only by addition of pharmacological doses of estrogens. However, the growth of tumors occurred 100 days after transplantation in the presence of 1 mg 17β-estradiol, suggesting the loss of dependency of MT6 tumors. Effects of various doses of 17β-estradiol on the growth of MT6 in orchiectomized rats (Group 6) are shown in Chart 3. Although the growth of MT6 was clearly inhibited again by 1 mg 17β-estradiol, treatments with 1 to 100 μg 17β-estradiol had no significant effects on the growth of MT6 tumors.

Histology of MT6 Tumors in Various Recipients. Tumors which developed in various recipients were classified into 3 types by predominant histological features: ductal carcinoma with cribriform pattern (Fig. 1); spindle cell carcinoma (Fig. 2); and mixed...
carcinoma of both types (Fig. 3). Ductal carcinoma in which tumor cells were arranged in a glandular pattern was the common feature of original MM. Spindle cell carcinoma was characterized by atypical proliferation of spindle-shaped cells without glandular structures. Mixed carcinoma was composed of both types of carcinomas. Metastases of spindle cell carcinomas were found in mediastinal and axillary lymph nodes, lungs, and kidneys, but no metastases of ductal carcinoma were found. Squamous metaplasia was observed in some metastatic lesions of spindle cell carcinoma in lymph nodes (Fig. 4).

Histology of MT6 tumors developed in orchiectomized rats (Group 2a) is shown in Chart 4. Tumors at early and intermediate stages were ductal carcinoma, whereas tumors at late stage were spindle cell carcinomas or mixed carcinomas. Histology of MT6 tumors developed in intact males (Group 1) showed a similar pattern (data not shown). Histology of MT6 tumors developed in orchiectomized rats receiving various doses of 17β-estradiol starting from the day of implantation (Groups 2a and 6) is shown in Chart 5. All small tumors developed in rats receiving 1 mg 17β-estradiol were spindle cell carcinomas, while all small tumors developed in rats without 17β-estradiol were ductal carcinomas. Seventy to 90 days after transplantation, spindle cell carcinoma and mixed carcinoma were commonly found in orchiectomized rats treated with 10 or 100 μg 17β-estradiol but not in those without 17β-estradiol (Chart 5).

Growth and ER of Ductal Carcinoma and Spindle Cell Carcinoma. The growth of 8 pure ductal carcinomas and 8 pure spindle cell carcinomas developed from MT6 was examined in intact and gonadectomized males given injections of or without 1 mg 17β-estradiol (Charts 6 and 7). Retransplanted ductal carcinoma could grow only in recipients without 1 mg 17β-estradiol, whereas retransplanted spindle cell carcinoma grew equally well in all recipients given injections with or without 1 mg 17β-estradiol.

Cytosol ER were demonstrated in all ductal carcinomas. In contrast, no measurable ER were found in any of the spindle cell carcinomas examined (Table 1).

DISCUSSION

We have reported that MM and MT6 are ovary-independent and hormone-independent tumors although they contain ER and...
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Figure 1. Growth of pure spindle cell carcinoma retransplanted in intact or orchiectomized rats either with or without 1 mg 17β-estradiol (E2) at 2-day intervals starting from the day of retransplantation. Seed tumors (spindle cell carcinoma) were obtained from orchiectomized rats given injections of 1 mg 17β-estradiol 130 to 170 days after transplantation of MT6. There were no significant differences among the growths of spindle cell carcinoma in 4 groups. Points, mean of 8 tumors; bars, S.D.

Table 1

<table>
<thead>
<tr>
<th>Tumor</th>
<th>ER level (fmol/mg cytosol protein)</th>
<th>Kd (x 10^-10 M)</th>
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<tr>
<td>Ductal carcinoma&lt;sup&gt;a&lt;/sup&gt; (5)</td>
<td>11.2 ± 4.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.82 ± 0.23</td>
</tr>
<tr>
<td>Spindle cell carcinoma&lt;sup&gt;d&lt;/sup&gt; (5)</td>
<td>&lt;2.0</td>
<td>Unable to calculate</td>
</tr>
</tbody>
</table>

<sup>a</sup> Ductal carcinoma responsive to 1 mg 17β-estradiol was obtained as described in “Materials and Methods.”

<sup>b</sup> Numbers in parentheses, number of tumors examined.

<sup>c</sup> Mean ± S.E.

<sup>d</sup> Spindle cell carcinoma unresponsive to 1 mg 17β-estradiol was obtained as described in “Materials and Methods.”

PGR, since MM and MT6 can grow equally well in male and female rats with or without gonadectomy (21). However, administration of pharmacological doses of estrogens clearly inhibited the growth of MT6 tumors (Charts 1 to 3). The growth of MT6 was unaffected by injection of CB-154 or LHM (Chart 1). The mechanism of tumor regression induced by high-dose estrogens in DMBA-induced mammary tumors in female rats has been thought to be due to decreased peripheral action of prolactin. This hypothesis is supported by the observations that administration of pharmacological doses of estrogens has been found to lower prolactin receptor content in the tumor (9) and that the effect of estrogens can be counteracted by administration of prolactin (13). The present data suggest that the mechanism of growth inhibition by high-dose estrogens in MT6 tumors is different. No inhibition in the growth of MT6 was observed by administration of sufficient amounts of CB-154 or LHM, which can inhibit secretion of pituitary prolactin markedly (2, 5, 14). The present observations suggest that the growth of MT6 is inhibited only by estrogen addition via an ER-related mechanism, although DMBA-induced mouse mammary tumors have been thought to be predominantly prolactin dependent (1, 20). However, a possibility that the effect of pharmacological doses of 17β-estradiol on tumor growth might be a toxic effect cannot be completely ruled out, since body weight of rats receiving 1 mg 17β-estradiol was approximately 20% lower than that of control animals or the animals receiving smaller doses of 17β-estradiol. It has been generally accepted that endocrine treatments such as both estrogen removal and addition of high-dose estrogens are effective in patients with ER-positive breast cancer (12, 15). However, the mechanism by which pharmacological doses of estrogen can inhibit growth of mammary cancer has not been elucidated. MT6 tumor is of some interest, since estrogen-withdrawal effects are dissociated from estrogen addition.

In human breast cancer, 30% of cancers are initially responsive to endocrine therapy, but eventually regrowth of hormone-independent tumors occurs (11). Kiang et al. (7) and Nomura et al. (16) observed in human breast cancer that a conversion of positive ER to negative ER occurred in about one-half of the initially endocrine-responsive tumors during endocrine therapy, although they did not observe that negative ER tumors changed to positive ER. In the MT6 tumor with ER, the loss of growth-inhibiting effect of pharmacological doses of estrogen occurred during high-dose estrogen treatment, since spindle cell carcinoma without ER developed in the tumor. MT6 seems to be a good model to investigate the mechanism involved in the loss of hormone dependency of cancer.

The present findings seem to show that the loss of growth-inhibiting effect of high-dose estrogens occurs by transformation of the cells from ductal carcinoma to spindle cell carcinoma with loss of ER. There are a few papers describing sarcomatous changes in transplantable adenocarcinoma in rats (3, 6, 10). Kitamura et al. (8) showed the transformation of androgen-dependent medullary carcinoma with androgen receptor into androgen-independent spindle cell carcinoma without receptor following androgen removal in mice, since these 2 types of cells had 6 identical chromosome abnormalities. The spindle-shaped cells found in MT6 tumors are thought to be of similar epithelial origin, because they showed squamous metaplasia in metastatic lesions (Fig. 4), although findings on original spindle cell tumors by electron microscopy gave no definite epithelial characteristics (data not shown). Future study is needed in order to clarify the origin of the spindle-shaped cells developed from MT6 tumor. Although Rous and Beard (17) and Foulds (4) introduced the general principles of cancer progression, there have been rather few good models. The spindle cell carcinoma seems to be a good model for the investigation of cancer progression.

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REFERENCES


Fig. 1. Histological feature of ductal carcinoma in orchiectomized rats on the 80th day after transplantation of MT6. H & E, x 120.

Fig. 2. Histological feature of spindle cell carcinoma in orchiectomized rats on the 150th day after transplantation of MT6. H & E, x 200.

Fig. 3. Histological feature of mixed carcinoma in orchiectomized rats on the 150th day after transplantation. Note the mixture of ductal carcinoma and spindle cell carcinoma. H & E, x 120.

Fig. 4. Metastatic tumor in mediastinal lymph node. Note squamous metaplasia of spindle cell carcinoma. H & E, x 120.
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