Growth-stimulating Effect of Pharmacological Doses of Estrogen on Androgen-dependent Shionogi Carcinoma 115 in Vivo but not in Cell Culture

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ABSTRACT

Shionogi carcinoma 115 (SCI15) had been accepted for 20 years as an androgen-dependent mouse mammary tumor, the growth of which is stimulated only by androgen. However, we very recently found that the growth of SCI15 tumors in vivo is stimulated not only by physiological doses of androgen but also by pharmacological doses of estrogen through the estrogen receptor system. In the present study, the growth-stimulative effect of estrogen on an androgen-dependent cloned cell line (SC-3) derived from SCI15 cells, which showed androgen- and estrogen-dependent growth in vivo, was examined in vitro. In serum-supplemented medium (Eagle's minimum essential medium containing 2% steroid-free fetal calf serum), testosterone or 5α-dihydrotestosterone (10^-8 - 10^-4 M) significantly stimulated the growth of SC-3 cells (3.2-fold increase in cell number at day 10 in culture containing 10^-4 M androgens) and changed the shape of SC-3 cells from epithelial to spindle (fibroblast-like), whereas 17β-estradiol (10^-10 - 10^-4 M) even in high concentrations had no such effects on SC-3 cells. Contrary to the effect of 17β-estradiol in vivo, 17β-estradiol as well as cyproterone acetate (10^-4 - 10^-4 M) inhibited the growth-stimulative effect of testosterone (10^-8 M) on SC-3 cells in a dose-dependent manner in the serum-supplemented medium. The anti-androgen and 17β-estradiol also showed comparable competitive effects on [3H]testosterone binding to androgen receptor in SC-3 cells. In serum-free medium [Ham's F-12:Eagle's minimum essential medium (1:1; v/v) containing 0.2% bovine serum albumin], testosterone (10^-9 M) also markedly stimulated the growth of spindle-shaped SC-3 cells, and epidermal growth factor (1 ng/ml) enhanced the growth-stimulative effect of testosterone, whereas 17β-estradiol (10^-9 - 10^-5 M) in the absence or presence of epidermal growth factor had no growth-stimulative effect on SC-3 cells. We conclude that the growth of SCI15 cells is stimulated by either physiological doses of androgen or pharmacological doses of estrogen in vivo but only by androgen in cell culture.

INTRODUCTION

Androgen-dependent mouse mammary carcinoma, SCI15, was established in 1964 by Minesita and Yamaguchi (1). SCI15 has maintained androgen dependency for growth both in vivo (2-4) and in cell culture (5-8) for more than 20 years and has been used for elucidating the mechanisms of the growth-stimulative action of androgen. On the other hand, the effect of estrogen on the growth of SCI15 tumors had not been studied in detail, since SCI15 tumors fail to grow in normal adult female mice, i.e., physiological concentrations of estrogen are ineffective for the growth of SCI15 tumors.

However, we recently examined the effect of various doses of estrogen on the growth of SCI15 tumors in vivo and obtained the following results. SCI15 tumor has ER as well as AR (9). The growth of SCI15 tumor is stimulated not only by physiological doses of androgen but also by pharmacological doses of estrogen, and androgen and estrogen synergize to stimulate the tumor growth (10, 11). The action of pharmacological doses of estrogen is not mediated via the AR system in SCI15 tumor, since the growth-stimulative effect of estrogen could not be inhibited by concomitant injections of anti-androgen such as cyproterone acetate (11). SCI15 tumors grown with physiological doses of androgen and pharmacological doses of estrogen are identical, since the high growth rate, histological type, maximum binding sites and Kd of ER and AR, pattern of secreted proteins from tumor cells in primary culture, and androgen dependency of tumors grown by androgen or estrogen were similar (11). These results show that both androgen and pharmacological doses of estrogen, although they elicit their growth-stimulative actions through different receptor systems, stimulate the growth of SCI15 tumors in vivo. By using an in vitro culture technique, however, Desmond et al. (5) and King et al. (6) reported that 17β-estradiol (10^-8 - 10^-5 M) even in high concentrations does not stimulate the proliferation of SCI15 cells in serum-supplemented medium. The SCI15 cells used by us (9-11) and others (5, 6) had been maintained for many years in vivo and in vitro, respectively. These findings suggest that the discrepancy in the growth-stimulative effect of estrogen on the SCI15 cells observed by us (9-11) and other investigators (5, 6) may be due to the difference in experimental conditions (in vivo and in vitro) or SCI15 cells used by us and others. In order to reply to the question and examine molecular mechanisms of androgen and estrogen actions on the growth of SCI15 cells, we established an androgen-dependent cell line (SC-3) derived from SCI15 tumor, which showed androgen- or estrogen-dependent growth in vivo, and examined the effects of androgen and/or estrogen on the growth of SC-3 cells in serum-supplemented and serum-free media in the present study.

MATERIALS AND METHODS

Animals and Tumors. Two- to 3-month-old male DS mice raised in our laboratory were used. When tumors were grafted in castrated mice, the castration was carried out at least 1 week in advance. A fragment of tumor (1 μl) was inserted beneath the dorsal skin, using a specially devised needle (1). Seed tumors of SCI15 were obtained from generations 333 to 350. The SCI15 tumors were maintained in male DS mice.

Chemicals. 17β-[1,2,6,7-3H]Estradiol (93 Ci/mmol), [1,2,6,7-3H]testosterone (99 Ci/mmol), and [1,2,6,7-3H]DHT (40 Ci/mmol) were obtained from New England Nuclear (Boston, MA). Nonradioactive steroids were from Steraloids, Inc. (Wilton, NH). The other chemicals were of analytical grade.

Injections of Estrogens. 17β-Estradiol and TP were suspended in 0.05 ml of vehicle (saline, 0.4% Polysorbate 80, 0.5% carboxymethylcellulose, and 0.9% benzyl alcohol) and were injected s.c. Control mice received injections of 0.05 ml of vehicle.

Determination of Tumor Growth. Length and width of each tumor...
cells with phosphate-buffered saline containing 0.02% EDTA and the medium was changed every other day. 4 weeks, or castrated mice bearing SCI 15 tumors given daily injections of 17β-estradiol (4 ng/mouse/day) starting from the day of transplantation. Tumor size (mean of length and width) was measured once a week, and the mean of the length and width was used as an index of tumor size.

Primary Cell Culture and Cloning. SCI 115 tumors grown in normal males were removed under sterile conditions 4 weeks after the transplantation of seed tumors. In order to obtain the dispersed cells, the minced tumors were digested with Hanks' balanced salt solution (Ca²⁺ and Mg²⁺ free, 10 ml/g tumor) containing trypsin (Worthington, 0.1 mg/ml), collagenase (Sigma type IV, 0.5 mg/ml), BSA (1 mg/ml), and N-2-hydroxyethylpiperazine-N'-ethanesulfonic acid (5 mM) for 30 min at 37°C, as reported previously (11). The mixture was then centrifuged at 300 x g for 5 min, and the pellet was washed once with 10% FCS-MEM supplemented with 2 mM glutamine and 60 μg/ml kanamycin to inhibit the activity of trypsin. The pellet was then washed 3 times with Hanks' balanced salt solution to remove cell debris. These dispersed cells in the pellet were resuspended in 2 ml of 10% FCS-MEM containing testosterone (10⁻⁴ M) and plated onto 35-mm dishes (1 x 10⁵ cells/dish). Cells were passaged once a week in the medium. The growth of cells was also found to be maintained in a medium composed of 2% FCS-MEM containing 10⁻⁴ M testosterone. After 20 passages, the cells were cloned by the limiting dilution method. The cloned cell lines obtained were cultured continuously in a maintenance medium composed of MEM containing testosterone (10⁻⁴ M) and 2% DCC-treated FCS. The DCC-treated FCS was obtained by incubating twice with charcoal at a final concentration of 1% at 37°C for 30 min, in order to remove endogenous steroids. Dextran T-70 (0.01%) was also added. SC-3 cells (one of the cloned cell lines obtained) were used in the following experiments. Cells were grown in a humidified incubator in 5% CO₂ at 37°C.

Cell Growth Experiments. To examine the effects of testosterone, DHT, 17β-estradiol, or testosterone in the presence of 17β-estradiol or cyproterone acetate on the growth of cells, SC-3 cells were routinely plated onto 35-mm dishes (approximately 1 x 10⁶ cells/dish) containing 2 ml of MEM added with 2% DCC-treated FCS and testosterone (10⁻⁴ M). On the following day [day 0], the medium was changed to an experimental medium composed of serum-supplemented medium [MEM containing 2% DCC-treated FCS], MEM containing 2–20% serum from castrated mice treated with or without high dose of 17β-estradiol, or serum-free medium [Ham's F-12:MEM (1:1, v/v) containing 0.2% BSA], with or without testosterone, DHT, 17β-estradiol, or testosterone in the presence of 17β-estradiol or cyproterone acetate. Steroids dissolved in ethanol solution were added to the medium, in which the final concentration of ethanol was 0.1%. Control dishes also contained 0.1% ethanol. Mouse serum was collected from castrated mice, castrated mice given daily injections of 100 μg 17β-estradiol for 4 weeks, or castrated mice bearing SCI115 tumors given daily injections of 100 μg 17β-estradiol for 4 weeks starting from the day of transplantation (30 mice/group). Blood was taken 12 h after the last injection. The medium was changed every other day.

Cell Harvest and Cell Count. Cells grown were harvested by washing the dishes once with phosphate-buffered saline and then incubating the cells with phosphate-buffered saline containing 0.02% EDTA and 0.05% trypsin for 15 min at 37°C. Two aliquots from each sample were counted in a hemocytometer.

ER and AR in Tumor Cytosols and Cultured Cells. SCI115 tumors grown in males were removed 24 h after the castration. ER and AR in tumor cytosols were assayed as described previously in our papers (9–11). The maximum binding sites and Kd were calculated according to the procedure of Scatchard (12). When we examined AR and ER in cytosols of SC-3 cells and inhibition of [3H]testosterone binding to AR by nonradioactive competitors in the SC-3 cells cultured in MEM added with 2% DCC-treated FCS and testosterone (10⁻⁴ M), the medium was changed to testosterone-free medium (MEM containing 2% DCC-treated FCS) 24 h prior to the harvest. When AR and ER in cytosols were measured, cells were harvested mechanically with a rubber policeman and resuspended (1 x 10⁶ cells/ml) in a buffer (10 mM Tris, 1.5 mM EDTA, 0.5 mM dithiotreitol, and 10 mM sodium molybdate, pH 7.4, at 20°C). The cells were homogenized in a glass Dounce homogenizer ( Kontes) using a B pestle, until more than 90% of the cells were disrupted (approximately 50 strokes), judged by a phase microscopy. The homogenate was centrifuged at 105,000 x g for 60 min and the supernatant was used as cytosol. ER and AR in the cytosol were assayed as described previously (9–11). When the inhibition of [3H]testosterone binding to AR by competitors in SC-3 cells was examined, SC-3 cells were harvested by incubating the cells with phosphate-buffered saline (Ca²⁺ and Mg²⁺ free) containing 0.02% EDTA and 0.05% trypsin for 15 min at 37°C, and the cells were washed with 10% FCS-MEM. The washed cells suspended in MEM containing 0.1% BSA (10⁵ cells/tube) were incubated with 10⁻⁴ M [3H]testosterone in the presence or absence of 10⁻⁴–10⁻⁶ M testosterone, 17β-estradiol, or cyproterone acetate in a humidified incubator for 60 min at 37°C. After the incubation, the cells were washed three times with MEM containing 0.1% BSA. Three ml of scintillation fluid (Atomlight; New England Nuclear) were decanted into the tube containing the incubated cells. After vortexing, the mixture was transferred into the counting vial. This procedure was repeated again, and radioactivity was measured.

Miscellaneous Assays. The protein concentrations were determined according to the method of Lowry et al. (13).

RESULTS

Stimulative Effects of Various Doses of TP, 17β-Estradiol, or Both on Tumor Growth in vivo. Seed SCI115 tumors were transplanted into castrated male mice and the mice were given daily injections of various doses of TP, 17β-estradiol, both, or vehicle starting from the day of transplantation. The growth of tumors was stimulated by TP or 17β-estradiol in a dose-dependent manner (Fig. 1, top and middle), and 17β-estradiol (4 μg/mouse/day) could act synergistically with TP (10 μg/mouse/day) to stimulate the growth of SCI115 tumors (Fig. 1, bottom). In the following experiments, we used a cloned cell line (SC-3) derived from the androgen- and estrogen-dependent SCI115 tumor to examine the growth-stimulative effects of androgen, estrogen, or both on SCI115 cells in vitro.

Effects of Androgens, Estrogen, or Both on Growth of SC-3 Cells in vitro. SC-3 cells were plated onto 35-mm dishes containing 2 ml of MEM added with 2% DCC-treated FCS and testosterone (10⁻⁴ M). On the following day (day 0), the medium was changed to MEM containing 2% DCC-treated FCS with or without various concentrations of testosterone (10⁻¹⁰–10⁻⁶ M), DHT (10⁻¹⁰–10⁻⁶ M), 17β-estradiol (10⁻¹²–10⁻⁶ M), or testosterone plus 17β-estradiol or cyproterone acetate. The medium was changed every other day, and the cells were harvested on day 10 in culture (Figs. 2, 5, and 6). Testosterone

![Graph](https://via.placeholder.com/150)

Fig. 1. Effects of various doses of androgen, estrogen, or both on the growth of SCI115 tumors in vivo. SCI115 seed tumors were transplanted into castrated male mice and the mice were given daily injections of TP (10–100 μg/mouse/day), 17β-estradiol (Et, 1-100 μg/mouse/day), or TP (10 μg/mouse/day) plus 17β-estradiol (4 μg/mouse/day) starting from the day of transplantation. Tumor size (mean of length and width) was measured once a week. Each point is the mean of 8–10 mice; bars, SE.
added in the medium stimulated the growth of SC-3 cells in a concentration-dependent manner, and the growth of cells reached a plateau at the concentration of $10^{-8}$ M. DHT added in the medium appeared to be more effective than testosterone at lower concentrations ($10^{-10}$-10$^{-8}$ M) but not at higher concentrations ($10^{-8}$-10$^{-6}$ M) (Fig. 2). The cells grown in the medium containing $10^{-3}$-10$^{-6}$ M of testosterone or DHT showed a fibroblast-like appearance (spindle) (Fig. 3), but the cells grown in the medium containing no or $10^{-10}$ M testosterone showed an epithelial appearance (Fig. 4). 17β-Estradiol had no effect on the growth or shape of SC-3 cells even at high concentrations (Fig. 5). When both testosterone ($10^{-8}$ M) and 17β-estradiol ($10^{-7}$-10$^{-6}$ M) were added to the medium, 17β-estradiol inhibited the testosterone-induced growth of cells in a concentration-dependent manner, probably due to a competitive inhibition of testosterone binding to AR by 17β-estradiol. The suggestion was supported by similar effects of cyproterone acetate (anti-androgen) and 17β-estradiol on the inhibition of testosterone-induced growth of SC-3 cells at various concentrations (Fig. 6). Furthermore, the suggestion was strongly supported by the findings that relative competitive effects of various doses of cyproterone acetate and 17β-estradiol on [3H]testosterone ($10^{-8}$ M) binding to AR in the cells were similar to the relative competitive effects on the androgen-induced growth of the cells (Figs. 6 and 7). The growth stimulation and testosterone binding to AR by $10^{-8}$ M testosterone were almost completely inhibited by $10^{-6}$ M 17β-estradiol or cyproterone acetate. The concentrations of 17β-estradiol or cyproterone acetate required to compete for 50% testosterone-induced growth and the total AR sites in SC-3 cells were found to be similar ($10^{-7}$-$10^{-6}$ M) in the presence of $10^{-8}$ M testosterone in culture medium (Figs. 6 and 7).

Effects of Androgen or Estrogen on Cell Growth in Serum-free Medium. The effect of androgen on the growth of SC-3 cells in serum-free medium was studied in order to rule out the influences of unknown factors included in the serum (FCS). SC-3 cells were plated onto 35-mm dishes (1 x 10$^4$ cells/dish) containing 2 ml of MEM added with 2% DCC-treated FCS and testosterone ($10^{-8}$ M). On the following day (day 0), the medium was changed to serum-free medium composed of Ham’s F-12:MEM (1:1, v/v) containing 0.2% BSA with or without testosterone ($10^{-8}$ M), insulin (1 µg/ml; Eli Lilly and Co.,
Indianapolis, IN), EGF (1 ng/ml; Collaborative Research, Waltham, MA), or combinations of them. The cells were harvested every other day.

In the serum-free medium without testosterone (Ham's F-12:MEM and 0.2% BSA with or without insulin or EGF), the growth of SC-3 cells could be maintained only until 4–6 days in culture, and the cells began to die thereafter, resulting in a decrease in cell number. On the other hand, in the serum-free medium added with testosterone, the proliferation of SC-3 cells could be maintained throughout the experimental period (12 days) and the cells grown were fibroblast-like in appearance. The addition of EGF significantly enhanced the testosterone-induced growth of SC-3 cells (Fig. 8).

In a separate experiment, the effect of 17β-estradiol on the growth of SC-3 cells was examined in the serum-free medium. On day 12 of culture, the numbers of cells grown in the serum-free medium (Ham's F-12:MEM) containing no, 10⁻⁸, 10⁻⁷, and 10⁻⁶ M 17β-estradiol were 2.9 ± 0.2, 3.0 ± 0.2, 2.9 ± 0.2, and 3.1 ± 0.1 x 10⁴ cells/dish (mean ± SE, n = 4), respectively. No effect of 17β-estradiol on the growth of SC-3 cells in the serum-free medium was demonstrated. The effect of 10⁻⁸ M 17β-estradiol when added with EGF (1 ng/ml) was also found to be negative for the growth of SC-3 cells (data not shown). The shape of cells grown in the presence and absence of 17β-estradiol was epithelial.

ER and AR in Cytosols from Tumors or Cultured Cells. ER and AR in cytosols from SC-115 tumors grown in normal males or from cultured SC-3 cells grown in MEM added with 2% DCC-treated FCS and testosterone (10⁻⁴ M) were examined. Significant differences in levels and Kₐ of ER and AR were not found between tumors grown in vivo and cells proliferated in vitro (Table 1).

Effects of Serum from Castrated Mice Treated with or without Pharmacological Dose of 17β-Estradiol on Growth of SC-3 Cells in Vitro. The present findings demonstrated that while both pharmacological doses of 17β-estradiol and physiological doses of androgen stimulate the growth of SC-115 tumors in vivo, only the androgen is able to stimulate the growth of the cells in vitro despite the presence of both ER and AR in the cultured cells. Since it is possible that the pharmacological doses of 17β-estradiol induce growth factor(s) in vivo from some tissue(s) which can stimulate the growth of SC-115 cells in the animals, the effect of serum from castrated mice treated with or without a pharmacological dose of 17β-estradiol on the growth of SC-3 cells in vitro was examined. However, the addition of various concentrations of serum (2–20%, v/v) from 17β-estradiol-
treated castrated mice with or without SC115 tumors resulted in no significant enhancement in the growth of SC-3 cells in culture, compared with that in the presence of serum from nontreated castrated mice (Table 2).

DISCUSSION

Target tissues for androgen or estrogen usually possess both AR and ER. Although androgen and estrogen act mainly in so-called androgen and estrogen target tissues, respectively, a lot of evidence has been accumulated to demonstrate that androgen elicits its effects in the estrogen target tissues (14–17) and estrogen does so in the androgen target tissues (18–21). Therefore, the mechanisms of interaction of androgen and estrogen in target tissues of sex steroids seem to be a very interesting object of study. The actions of androgen in the estrogen target tissues seem to depend on a dose of androgen. Physiological doses of androgen inhibit estrogen-induced protein synthesis, weight gain in the uterus (14), and the growth of dimethylbenzanthracene-induced rat mammary tumor (15). However, pharmacological doses of androgen stimulate progesterone receptor synthesis, weight gain in the uterus (16), the growth of dimethylbenzanthracene-induced rat mammary tumor (15), and the proliferation of MCF-7 cells (17). It has been demonstrated that pharmacological doses of androgen act as an estrogen agonist through ER but not AR in the estrogen target tissues, because ER-androgen complexes formed by pharmacological doses of androgen and ER-estrogen complexes have been shown to elicit almost the same biological and biochemical responses in the estrogen target tissues (15–17). On the other hand, the actions of estrogen in the androgen target tissues are quite different from those of androgen in the estrogen target tissues. Moore et al. (18) have demonstrated that physiological doses of estrogen increase AR contents in the dog prostate and enhance the androgen activity in the prostate. The induction of AR by estrogen has also been demonstrated in the prostate (19) and the uterus (20) of rats. Physiological doses of estrogen seem to act through ER but not AR in the so-called androgen target tissues. There appears to be no report to show that pharmacological doses of androgen clearly elicit androgenic activities in the estrogen target tissues. We found that physiological doses of androgen and pharmacological doses of estrogen show the same stimulative in vivo effect on the growth of SC115 tumor having both AR and ER (Ref. 11; Fig. 1). The action of pharmacological doses of estrogen in vivo was shown not to be mediated via the AR system in the SC115 tumor (11). Furthermore, high concentrations of 17β-estradiol inhibited the androgen-induced growth of the cells in culture competing for AR (Figs. 6 and 7). The finding is consistent with previous observations that AR-estrogen complexes formed by pharmacological doses of estrogen are biologically inactive. Therefore, pharmacological doses of estrogen seem to stimulate the growth of SC115 tumors through ER but not AR, although the estrogen activity may be induced by ER present in some of nontransformed cells. The SC115 tumor seems to be a very unique model for elucidating the mechanism of estrogen activity in the growth of androgen-dependent tissues and tumors.

By using an in vitro culture technique, Desmond et al. (5), King et al. (6), and Yates and King (22) found that 17β-estradiol even in very high concentrations did not stimulate the proliferation of cloned SC115 cells in serum-supplemented medium and that the shape of SC115 cells changed from fibroblast-like to epithelial when androgen was removed from the medium. Although the morphological change of SC115 cells induced by us in vivo by androgen depletion or deficiency was inverse (medullary carcinoma (epithelial) to spindle-shaped cells (fibroblast-like)) (3, 10, 11), a cloned androgen-dependent cell line (SC-3 cells) derived from the same SC115 cells changed from fibroblast-like to epithelial when androgen was removed in culture in the present study. Furthermore, high concentrations of 17β-estradiol did not stimulate the proliferation of SC-3 cells in the serum-supplemented and serum-free media in the present study, whereas the growth of SC115 tumors used by us was stimulated by high doses of estrogen in vivo (11). Therefore, the present findings in culture seem to demonstrate that the discrepancy in estrogen-dependent growth and changes of the morphology of the SC115 cells observed previously by us (3, 11) and other investigators (5, 6, 22) seems to be due to the difference in the experimental conditions (in vivo and in vitro) used. In vivo experiments, testosterone was less effective than DHT in stimulating the growth of SC115 tumors when two were injected in equal doses (10, 23). In cytosol of SC115 tumors, the binding affinity for DHT was approximately 3 times higher than that for testosterone (3). In cell culture of SC115 cells, DHT appeared to be effective at concentrations about 5 times lower than testosterone (5). These previous findings by us (3, 10) and others (5, 23) are not inconsistent with the present observations that DHT added in culture medium appeared to be more effective than testosterone at lower concentrations (10^-16–10^-9 M) in stimulating the growth of SC-3 cells in vitro, although the difference in the growth-stimulative effect between DHT and testosterone was less marked in the present findings shown in Fig. 2.

Recent studies have shown that the stimulative effect of sex steroids on cell proliferation may be mediated by specific polypeptide growth factor(s) (21). If the growth-stimulative effect of androgen on SC115 cells is mediated by such factor(s), the SC115 cells themselves may produce growth factor(s) (autocrine control), since the proliferation of cloned SC115 cells in serum-supplemented (Refs. 5, 6, and 8; Fig. 2) and serum-free media (Fig. 8) has been shown to be augmented by physiological concentrations of androgens. On the other hand, pharmacological doses of estrogen may not stimulate the growth of SC115 cells by autocrine control mechanism, because the growth of SC115 cells has been shown to be stimulated by high doses of estrogen in vivo (Ref. 11; Fig. 1) but not in vitro under the conditions used (Refs. 5 and 6; Fig. 5). The lack of estrogen responsiveness of the SC115 cells in culture may not be due to the loss of ER in the cells, since SC-3 cells in culture and SC115 cells in vivo contained almost the same level of ER with similar.

Table 2 Growth of SC-3 cells in medium supplemented with various concentrations of serum obtained from castrated DS mice treated with or without pharmacological doses of 17β-estradiol

<table>
<thead>
<tr>
<th>Serum from</th>
<th>2%</th>
<th>5%</th>
<th>10%</th>
<th>20%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castrated males</td>
<td>3.3 ± 0.2*</td>
<td>5.2 ± 0.3</td>
<td>6.2 ± 0.3</td>
<td>6.4 ± 0.3</td>
</tr>
<tr>
<td>Castrated males treated with 17β-estradiol</td>
<td>3.6 ± 0.2</td>
<td>5.6 ± 0.3</td>
<td>6.5 ± 0.3</td>
<td>6.7 ± 0.4</td>
</tr>
<tr>
<td>Castrated males bearing tumors treated with 17β-estradiol</td>
<td>3.7 ± 0.2</td>
<td>5.5 ± 0.4</td>
<td>6.5 ± 0.3</td>
<td>6.9 ± 0.3</td>
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*Mean ± SE of 4 dishes.
affinities (Table 1). Since high concentrations of 17β-estradiol inhibited the testosterone-induced growth of cloned SC-3 cells in vitro (Fig. 6) by competing for AR (Fig. 7), the synergistic activity of high doses of 17β-estradiol with testosterone in vivo seems to be mediated via growth factor(s) produced in some of nontransformed cells (paracrine or endocrine control). It is possible to speculate that the estrogen-induced stimulation by paracrine or endocrine control may be greater than the estrogen-induced inhibition of androgen-dependent autocrine control. However, the addition of serum from castrated mice treated with high dose of 17β-estradiol resulted in no significant enhancement in the growth of SC-3 cells in culture (Table 2). The possible mechanisms to explain different effects of estrogen in vivo versus in vitro concerning mitogenic properties seem to be interesting and should be examined in future studies using this model system. Since all sera examined were taken 12 h after the last injection from castrated mice chronically treated by injection of 17β-estradiol (Table 2), stimulative effects of factor(s) on the growth of SC-3 cells in culture should be carried out using sera and tissue extracts taken under various conditions. However, the possibility that the growth of tumors in vivo is indeed due to growth factor(s) produced by the SC115 cells, the synthesis of which is impaired in vitro, must be taken into account.

In the present study, the growth-stimulative effect of testosterone (10⁻⁸ M) on a cloned cell line (SC-3 cells) derived from SC115 cells was clearly demonstrated in serum-free medium containing only 0.2% BSA. Furthermore, the shape of SC-3 cells changed from epithelial to fibroblast-like when testosterone was added in the serum-free medium, as was observed in serum-supplemented medium. The addition of EGF enhanced the testosterone-induced growth of SC-3 cells in the serum-free medium, whereas EGF alone had only a small stimulative effect. The testosterone-induced growth of SC-3 cells in the serum-free medium seems to be a good model for elucidating molecular mechanisms of androgen enhancement in the growth of androgen-dependent tissues and tumors.

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