Androgen Dependency of a Tumor Produced by a Cell Line Derived from Androgen-responsive Shionogi Carcinoma 115

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ABSTRACT

An androgen-dependent tumor (SCC8 tumor) was obtained by inoculating an androgen-responsive cell line derived from the androgen-responsive Shionogi carcinoma 115 (SC115) into mice and then treating the mice with testosterone propionate (TP) at a pharmacological dose (400 μg/day). The SCC8 tumor differed in histological appearance from the SC115 tumor and its growth was less stimulated by androgen than that of the SC115 tumor. However, its growth was completely androgen dependent; SCC8 tumors did not develop in castrated mice and regressed when TP treatment was discontinued. The decreased sensitivity of the SCC8 tumor seemed to be attributable in part to its rapid metabolism of testosterone metabolites with lower androgenic actions. The effects of TP at doses of 0, 100, 200, and 400 μg/day on cell division and cell death in SCC8 tumors of medium size were examined by measurements of the mitotic index and the retention of 5-[125I]iodo-2'-deoxyuridine incorporated into the whole tumor. TP increased the mitotic index dose dependently and at all doses reduced the decrease in the retention of 5-[125I]iodo-2'-deoxyuridine. These results suggest that steroids may not only stimulate cell division but also reduce cell death in steroid-dependent tumors.

INTRODUCTION

Shionogi carcinoma 115 is a mouse mammary carcinoma with the histological features of an undifferentiated medullary carcinoma (1, 2). Because this SC115 tumor shows androgen-responsive growth, it has been used as a model of steroid-dependent tumors (2-5). SC115 cells (medullary carcinoma cells) show androgen-responsive growth, but on depletion of androgen, they easily change to spindle-shaped cells or chondroid cells which show androgen-independent growth (6-8). Thus, when SC115 cells are inoculated into castrated mice, tumors composed of spindle-shaped cells and chondroid cells which show androgen-independent growth (6-8). When SC115 cells are inoculated into castrated mice, tumors composed of spindle-shaped cells and chondroid cells develop and grow slowly (7), whereas tumors composed entirely of the original SC115 cells develop in intact male mice or castrated mice given androgen (2, 6-8). The result of depletion of androgen at a late stage of tumor growth is, however, rather complex; namely, the tumors continue to grow with only temporary or no regression (6, 8), and they become composed of androgen at a late stage of tumor growth is, however, rather complex; namely, the tumors continue to grow with only temporary or no regression (6, 8), and they become composed of spindle-shaped cells and chondroid cells which show androgen-independent growth (6-8). Thus, the growth of the tumor that develops after inoculation of SC115 cells does not necessarily depend on androgen.

Recently, we obtained an androgen-dependent tumor by inoculating a cell line derived from the SC115 tumor into castrated DS mice and then treating the mice with androgen at a pharmacological dose. This tumor did not develop in castrated mice and regressed after removal of androgen, but its growth was less sensitive to androgen than that of the SC115 tumor. In target organs such as the uterus, prostate, and seminal vesicle, steroids are known not only to stimulate cell division but also to prevent cell death (9-14). However, little is known about the effect of steroids on cell death in steroid-dependent tumors. Because the tumor produced by the cell line derived from the SC115 tumor exhibited complete androgen dependency, it seemed useful for the study of the effect of steroids on cell death in steroid-dependent tumors. This paper reports studies on the androgen dependency of this tumor. The results suggest that steroids may delay cell death as well as stimulate cell division in steroid-dependent tumors.

MATERIALS AND METHODS

Mice. PGK contains two isozymes (types A and B) which can be identified by starch gel electrophoresis (15-19). The PGK types are determined by the Pgk-1 gene which is linked with the X chromosome (15-19). Standard DS mice, which are maintained in our laboratories, have a Pgk-1* gene (male Pgk-1*/Y and female Pgk-1*/Pgk-1* mice) and show only type B PGK. We introduced a Pgk-1* gene into the DS strain and obtained a congenic strain showing type A PGK (male Pgk-1*/Y and female Pgk-1*/Pgk-1* mice) as described previously (7). Male DS-Pgk-1*/Y and DS-Pgk-1*/Y mice were used at 2 to 4 months of age. When castrated mice were used, the operation was carried out 1 week before experiments. No difference was found in the growths of tumors in male DS-Pgk-1*/Y and DS-Pgk-1*/Y mice, but unless otherwise mentioned, DS-Pgk-1*/Y mice were used.

Cells. The androgen-responsive cell line designated as SCC8 cells was obtained from a SC115 tumor grown in a male DS mouse as described previously (20) and was maintained in DMEM (Nissui Pharmaceutical Co., Tokyo, Japan) with 5% FCS containing 3 × 10^4 M testosterone.

Cell Growth in Monolayer Culture. SCC8 cells were suspended in DMEM with 2% FCS and plated at 2 × 10^4 cells/35-mm plastic tissue culture dish. On the following day, the medium was replaced by DMEM (3 ml) containing 2% FCS, with or without 3 × 10^4 M testosterone. The medium was changed every 3 days, and cells were harvested on day 12 of culture and counted in a hemocytometer.

Inoculation of SCC8 Cells Into Mice. SCC8 cells were cultured in DMEM with 5% FCS containing 3 × 10^4 M testosterone. The cells were harvested by treatment with phosphate-buffered saline (calcium and magnesium free; pH 7.2) containing 0.5% trypsin (Difco, 1,250; Difco Laboratories, Detroit, MI) and 0.02% EDTA and washed twice with DMEM. The washed cells were then suspended in DMEM at a concentration of 10^6 cells/ml and 0.5 ml of the suspension was injected s.c. into the neck of each mouse.

Transplantation and Growth of Tumors. Tumor tissues free of necrotic regions were cut into pieces approximately 2 mm^2 and one piece was implanted with a trocar into the interscapular s.c. tissue of each mouse (1). The length and width of the resulting tumors were measured every week and the mean 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: SC115, Shionogi carcinoma 115; TP, testosterone propionate; PGK, 3-phosphoglycerate kinase (EC 2.7.2.3); FCS, fetal calf serum; DMEM, Dulbecco's modified Eagle's medium; [125I]IDUrd, 5-[125I]iodo-2'-deoxyuridine.

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Injection of Steroids. Testosterone propionate, 17β-estradiol, or dexamethasone was suspended in 0.1 ml of vehicle (0.9% NaCl solution, 0.4% polysorbate 80, 0.5% carboxymethyl cellulose, and 0.9% benzyl alcohol) and injected into mice s.c.

Histology. Tissues were fixed in Zamboni's fixative (21) and embedded in paraffin. Sections 5 µm thick were cut and stained with hematoxylin and eosin.

Androgen Receptor in Tumor Cytosol. Tumors grown in castrated mice treated with 400 µg/day of TP were removed 48 h after the last injection of TP. Necrotic parts and connective tissue were carefully removed and the tumor tissue was homogenized with 5 volumes of 10 mm Tris-HCl (pH 7.4 at 20°C) containing 1.5 mm EDTA, 0.5 mm dithiothreitol, and 10 mm sodium molybdate, using a Polytron PT10 homogenizer (Brinkmann Instruments, Inc., Westbury, NY). The homogenate was centrifuged at 105,000 × g for 60 min. The supernatant cytosol was used for androgen receptor assay, and the pellet was used for DNA assay. The androgen receptor was assayed as described previously (22). The DNA content was determined by the method of Burton (23).

Metabolism of [3H]Testosterone in Tumors. The tumor was inoculated into castrated mice and the mice were then given TP daily at a dose of 400 µg/day for 3 weeks. Then, 48 h after the last injection of TP, a solution (0.1 ml) of [1,2,6,7-3H]testosterone (88 Ci/mmol; New England Nuclear, Boston, MA) at 60 µCi/0.1 ml in saline containing 5% ethanol was injected s.c. into tumor-bearing mice. The mice were sacrificed 1 h after the injection of [3H]testosterone and tumor tissue was carefully separated from necrotic parts and connective tissue. One part (200–250 mg) was homogenized with saline and the homogenate was used for DNA assay. Another part of the tumor tissue (500–1200 mg) was also homogenized with saline and radioactive steroids in the homogenate were separated by three extractions with 10 ml of diethyl ether. Then, the radioactive steroids were separated as described previously (24) and were recrystallized immediately or after acetylation with 15–20 mg of nonradioactive standard steroids (Steraroids, Inc., Wilton, NH) to constant specific activity in order to estimate their amounts accurately.

Androgen Receptor and Metabolism of [3H]Testosterone in Cells from Tumors. Cells were obtained from tumors as described previously (20) and cultured in DMEM with 5% FCS containing 3 × 10⁻⁴ M testosterone. These cells were subcultured and then used for experiments. For assay of androgen receptor, cells were cultured in medium without testosterone for 24 h and then whole cell assay was done by using mixtures of known amount of the isozymes as standards, as described previously (26) and were expressed as percentages of the total radioactivity recovered.

RESULTS

Androgen Dependency of the Tumor Produced by the Cell Line Derived from the SCI 15 Tumor. A cell line was obtained from the SCI 15 tumor by the limiting dilution method. This cloned cell line was designated as SCC8 cells. SCC8 cells were cultured at 2 × 10⁶ cells/dish in medium containing 2% FCS, with or without 3 × 10⁻⁸ M testosterone, and the number of cells per dish was counted after 11 days. The number of cells increased to 86.9 ± 10.4 × 10⁶ and 43.0 ± 1.2 × 10⁶ (n = 4) in cultures with and without testosterone, respectively, indicating androgen responsiveness of the SCC8 cells.

When 5 × 10⁷ SCC8 cells were injected into castrated and intact male mice, tumors appeared in some castrated and intact male mice, but their growths were very slow (data not shown). When castrated male mice were given the daily injections of TP at a pharmacological dose (400 µg/day) from immediately after cell inoculation, however, tumors developed and grew rapidly in all the mice (data not shown).

We designated the rapidly growing tumor as SCC8 tumor and maintained it by successive transplantations in castrated male mice treated daily with 400 µg/day of TP. The SCC15 tumor is an undifferentiated medullary carcinoma forming nests of compact cells, whereas the SCC8 tumor consists of cells with a large nucleus and prominent nucleolus that do not form cell nests but intermingle with host cells (Fig. 1). After 7 successive transplantations, the androgen responsiveness of the SCC8 tumor was compared with that of the SCC15 tumor (Fig. 2). The SCC8 tumor did not grow in castrated male mice that were not treated with TP, but it developed in some intact male mice and in all castrated mice treated with TP. Its growth was stimulated maximally by injection of TP at 400 µg/day. Its growth for 1–3 weeks after its inoculation into castrated mice was less than one-half as much after injection of TP at 100 µg/day than after injection of TP at 400 µg/day. The SCC15 tumor grew even in castrated male mice, although very slowly. Growth of the SCC15 tumor was markedly stimulated by TP; its growth for 1–3 weeks after its inoculation into castrated mice was maximal with TP at a dose of 400 µg/day and about three-fourths as great with TP at 100 µg/day (tumor sizes, 21.8 ± 1.8 versus 17.2 ± 1.1 mm at 2 weeks and 35.5 ± 2.1 versus 26.5 ± 2.5 mm at 3 weeks). The maximal growth rate of the SCC15 tumor was much greater than that of the SCC8 tumor. Injection of 17β-estradiol or dexamethasone at a dose of 100 µg/day did not inhibit endogenous thymidine synthesis, and 1 h later, 0.2 µCi/g body weight of [125I]IdUrd, 2200 Ci/mmol (New England Nuclear), was injected i.p. After treatment with [125I]IdUrd, the mice were divided into 5 groups. One group was killed after 4 h, and whole tumor was removed and placed in 10% phosphate-buffered formalin (pH 7.4). The mice in the other groups were given daily injections of vehicle only and 100, 200, and 400 µg/day of TP, respectively. Mice in these groups were killed on days 4, 9, and 13 after [125I]IdUrd injection, and their whole tumors were put into 10% phosphate-buffered formalin (pH 7.4). The 125I radioactivity retained in the tumors was determined as described previously (6, 26). The whole tumor was cut into small pieces and immersed in 10% buffered formalin (pH 7.4) for 7 days with daily changes of the formalin. The 125I radioactivity retained in the whole tumor was measured with an Auto-Gamma counter and expressed as a percentage of the total 125I radioactivity injected.

The 125I radioactivities in the RNA, DNA, and protein fractions were then determined as described previously (26) and were expressed as percentages of the total radioactivity recovered.
ANDROGEN-DEPENDENT TUMOR DERIVED FROM SCI

Fig. 1. Histological appearance of SCI IS and SCC8 tumors. A, SCI IS tumor. The tumor is composed of compact cells. B, SCC8 tumor. The tumor is composed of cells having a nucleus with a prominent nucleolus. Tumor cells are intermingled with host cells. H & E, x 500.

Fig. 2. Growth of SCC8 and SCI15 tumors. SCC8 (A) and SCI15 (B) tumors were inoculated into castrated and intact male mice. Castrated male mice received daily injection of TP at doses of 100, 200, and 400 μg/day (TP 100, TP 200, and TP 400). SCC8 tumors developed in 4 of 6 intact male mice and in all castrated male mice treated with TP but not in castrated male mice without TP injection. SCI15 tumors developed in all intact male mice and in all castrated male mice with or without TP injection. Points, mean sizes ± SE (bars) of tumors in 4–7 mice.

was transplanted into castrated male mice, which were given daily injections of TP at 400 μg/day for 2 or 3 weeks. When TP injection was stopped, the SCC8 tumors which had reached a mean tumor size of 13 or 18 mm gradually regressed and disappeared completely.

Difference in Sensitivities of SCC8 and SCI15 Tumors to Androgen. The amounts and dissociation constants of androgen receptors of the tumors and their metabolism of testosterone were examined. The dissociation constants of the androgen receptor of the SCC8 and SCI15 tumors were 0.19 ± 0.03 and 0.20 ± 0.03 nM (n = 4), respectively, which were not significantly different. However, the maximal number of binding sites of androgen receptors in the SCC8 tumor (8.4 ± 0.8 fmol/mg DNA; n = 4) was about 60% of that in the SCI15 tumor (14.8 ± 0.6 fmol/mg DNA; n = 4). For investigation of metabolism of testosterone in vivo, [3H]testosterone was injected into tumor-bearing mice and the amounts of C19 steroids in the tumors were determined 1 h later (Table 1). The average amount of total steroids extracted from 6 SCC8 tumors was 66% of that extracted from 5 SCI15 tumors (2110 ± 200 versus 3204 ± 254 pmol/100 mg DNA). The efficiency of extraction of steroids from tumor tissues was evaluated by determining the recovery of 4 × 10^5 dpm of [3H]testosterone (1 μCi/nmol) added to homogenates of tumor tissues (500 mg). The recoveries of [3H]testosterone from homogenates of SCC8 and SCI15 tumor tissues were 95.7 ± 0.2 and 96.8 ± 0.2% (n = 4) which were not significantly different. In both the SCC8 and SCI15 tumors, testosterone was the main steroid, followed by 5α-dihydrotestosterone, but the sum of the amounts of these two steroids in

not induce growth of the SCC8 tumor, but both stimulated growth of the SCI15 tumor, as described previously (20, 27). These results indicate that the SCC8 tumor is androgen responsive but that it is less responsive than the parent SCI15 tumor.

We examined the effect of removal of androgen during growth of the SCC8 tumor (Fig. 3). For this, the SCC8 tumor

![Fig. 3. Effect of androgen depletion on growth of SCC8 tumors. The SCC8 tumor was inoculated into three groups of castrated male mice and the mice were then given TP at 400 μg/day. TP injections were discontinued in one group each after 2 and 3 weeks (o) and were continued in the third group (C). Points, mean tumor sizes ± SE (bars) in 5–6 mice.](image)

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Amount of steroid (pmol/100 mg DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone</td>
<td>SCC8: 601 ± 80^a SCI15: 1401 ± 131</td>
</tr>
<tr>
<td>5α-Dihydrotestosterone</td>
<td>SCC8: 108 ± 18^b SCI15: 205 ± 14</td>
</tr>
<tr>
<td>4-Androstene-3,17-dione</td>
<td>SCC8: 33 ± 6 SCI15: 21 ± 1</td>
</tr>
<tr>
<td>5α-Androstane-3,17-dione</td>
<td>SCC8: 7 ± 1 SCI15: 10 ± 1</td>
</tr>
<tr>
<td>Androsterone</td>
<td>SCC8: UD SCI15: UD</td>
</tr>
<tr>
<td>5α-Androstane-3α,17β-diol</td>
<td>SCC8: 40 ± 8^c SCI15: UD</td>
</tr>
</tbody>
</table>

^a Mean ± SE of values in 6 SCC8 tumors and 5 SCI15 tumors.
^b Significant difference from the value for the SCI15 tumor (P < 0.05 by Student's t test).
^c UD, undetectable.
the SCC8 tumor was 44% of that in the SC115 tumor. In contrast, the total amount of other C19 steroids (shown in Table 1) was much greater in the SCC8 tumor than in the SC115 tumor (80 ± 16 versus 31 ± 1 pmol/100 mg DNA).

The SCC8 tumor differs histologically from the SC115 tumor (Fig. 1). Thus, it was conceivable that the proportions of contaminating host cells differed in preparations of the two tumor tissues for androgen receptor assay and for examination of the metabolism of testosterone. Therefore, we inoculated the SCC8 and SC115 tumors into castrated DS-Pgk-I'/'Y mice and examined the PGK patterns of tissues from the tumors developed in these mice. The PGK types of the SCC8 and SC115 tumor cells are type B since these tumors developed in DS-Pgk-I'/'Y mice which contain only type B PGK. Type A PGK was found to amount to less than 5% of the total PGK in 6 SC115 tumors and to 5-14% of the total in one SC115 tumor, whereas it constituted 25-34% of the total in 4 SCC8 tumors and 35-44% of the total in 12 SCC8 tumors. Thus, only about 60% of the cells in preparations of SCC8 tumors were in fact SCC8 tumor cells.

Androgen receptors were examined in cultured cells from SCC8 and SC115 tumors. Results showed that the receptor of SCC8 tumor cells has a dissociation constant similar to that of SC115 tumor cells (0.72 ± 0.09 versus 0.91 ± 0.21 nM; n = 4) but that there were more binding sites on SCC8 cells (4.16 ± 0.59 versus 1.48 ± 0.21 fmol/10^6 cells; n = 4).

We also studied the metabolism of testosterone in these cells in vitro. For this, cells were cultured in medium containing 2 nM of [3H]testosterone and then the amounts of 5α-dihydrotestosterone and less androgenic steroids such as 4-androstene-3,17-dione, 5α-androstane-3,17-dione, androsterone, and 5α-androstane-3α,17β-diol in the medium and the cells were measured. Cells from the SCC8 tumor produced more 5α-dihydrotestosterone (30.1 ± 1.9 versus 4.3 ± 0.8 pmol/10^6 cells; n = 5) and more of the less androgenic steroids than cells from the SC115 tumor (76.0 ± 8.1 versus 35.8 ± 2.3 pmol/10^6 cells).

Effects of Androgen on Division and Death of SCC8 Tumor Cells. The SCC8 tumor was inoculated into castrated mice and the mice were given 400 μg/day of TP for 2 weeks. Then, the mice were divided into 4 groups. TP injection was discontinued in one group and was continued at daily doses of 100, 200, and 400 μg, respectively, in the 3 other groups. On discontinuation of TP injection the SCC8 tumor gradually regressed, but in the other groups it continued to grow (Fig. 4). Doses of 200 and 400 μg/day of TP caused similar stimulation of growth, while TP at 100 μg/day induced slightly less growth.

We also investigated the effect of androgen on division and death of the SCC8 tumor cells in this experimental system. Tumor-bearing mice treated with vehicle only or with various doses of TP were killed on day 4, 9, or 13 after the start of treatment, and their mitotic and apoptotic indices were determined (Table 2). These indices were determined by examination of intact tumor tissue in the outer portion of tumors, inasmuch as the central portions were necrotic. The mitotic index was lowest in SCC8 tumors in mice not treated with TP (Table 2). TP at doses of 100 and 200 μg/day caused a dose-dependent increase in the mitotic index, and a dose of 400 μg/day caused an increase similar to that with 200 μg/day. In contrast, the apoptotic index was not significantly different in mice with and without TP injections at these doses (Table 2; Fig. 5).

The effect of androgen on death of SCC8 tumor cells was examined by measuring the retention of [125I]IdUrd incorporated into the tumor. For this, [125I]IdUrd was incorporated into the SCC8 tumor before treatment with vehicle or 100, 200, and 400 μg/day of TP, and the 125I radioactivity retained in the whole tumor was determined on days 4, 9, and 13 after the start of these treatments (Fig. 6). About 90% of the incorporated 125I radioactivity was found in the DNA fraction of the whole tumor (Table 3). When TP injection was discontinued, the 125I radioactivity retained in the whole tumor diminished rapidly, and continuation of TP injection at doses of 100, 200, and 400 μg/day did not prevent loss of 125I radioactivity. However, on day 13, when the size of tumors in mice not treated with TP began to decrease (Figs. 3 and 4), the 125I radioactivity retained in the whole tumor was significantly higher in the tumors in mice treated with 100, 200, or 400 μg/day of TP than in the tumors in mice treated with vehicle only. The retention of 125I radioactivity in tumors in mice treated with 100, 200, and 400 μg/day of TP did not depend on the dose of TP.

DISCUSSION

The androgen-responsive SCC8 cells formed androgen-dependent tumors (SCC8 tumors). These tumors differed from SC115 tumors in histological appearance, androgen dependency of growth, sensitivity to androgen, and responsiveness to 17β-estradiol and dexamethasone. Tumors are not homogeneous in their cell compositions but are composed of heterogeneous subpopulations (28-30). Cells obtained from individual tumors have been shown to differ in immunogenicity (31), growth rate (32), tumorigenicity (33), karyotype (34), metastatic potential (29, 30, 33), drug resistance (35), hormone receptors (36), and responsiveness to hormones (37). Furthermore, cloned cells have been found to generate heterogeneous subpopulations during growth in vitro or in vivo (28-30, 38). Thus, the differences between the SCC8 and SC115 tumors may be attributable to heterogeneous subpopulations in the SC115 tumor or to generation of heterogeneous subpopulations during growth of SCC8 cells.

The amount of androgen receptor in the SCC8 tumor was about 60% of that in the SC115 tumor. Because the amount of androgen receptor was expressed in fmol/mg DNA, it reflects the average receptor content of cells in the tumor tissue. Examination of proportions of tumor cells and host cells in the tumor tissues showed that only about 60% of the cells in most SCC8 tumors were SCC8 tumor cells, whereas almost all of the cells in SC115 tumors were SC115 tumor cells. Contaminating...
The SCC8 tumor was inoculated into castrated mice and the mice were then treated with TP at 400 µg/day. Two weeks after tumor inoculation, mice were divided into 4 groups. TP injection was discontinued in one group and was continued at doses of 100, 200, and 400 µg/day, respectively, in the other group. The mitotic and apoptotic indices were determined 4, 9 and 13 days later.

### Table 2: Effects of TP on the mitotic and apoptotic indices

<table>
<thead>
<tr>
<th>Dose of TP (µg/day)</th>
<th>Mitotic index (%)</th>
<th>Apoptotic index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 4</td>
<td>Day 9</td>
</tr>
<tr>
<td>0</td>
<td>0.39 ± 0.07</td>
<td>0.17 ± 0.04</td>
</tr>
<tr>
<td>100</td>
<td>1.19 ± 0.16</td>
<td>0.82 ± 0.09</td>
</tr>
<tr>
<td>200</td>
<td>2.79 ± 0.17</td>
<td>2.70 ± 0.31</td>
</tr>
<tr>
<td>400</td>
<td>3.05 ± 0.44</td>
<td>3.48 ± 0.54</td>
</tr>
</tbody>
</table>

* Mean ± SE of values in 5-6 tumors.

* Significant difference from value for tumors in mice without TP injection (P < 0.05 by Student's t test).

* Significant differences from values for tumors in mice without TP injection (P < 0.05 by Student's t test).

Table 3: 125I radioactivities in RNA, DNA, and protein fractions of SCC8 tumors with incorporated [125I]IdUrd

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Day 0</th>
<th>Day 4</th>
<th>Day 9</th>
<th>Day 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA</td>
<td>1.3 ± 0.1</td>
<td>1.7 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>DNA</td>
<td>91.1 ± 0.5</td>
<td>91.8 ± 0.4</td>
<td>90.3 ± 0.3</td>
<td>89.7 ± 1.3</td>
</tr>
<tr>
<td>Protein</td>
<td>7.6 ± 0.4</td>
<td>6.5 ± 0.4</td>
<td>7.7 ± 0.3</td>
<td>8.8 ± 1.0</td>
</tr>
</tbody>
</table>

* Mean ± SE of values in 5-7 tumors.

Fig. 5. An apoptotic cell (arrow) in the SCC8 tumor. H & E, × 1250.

Fig. 6. Effect of TP on the retention of 125I radioactivity in whole tumors with incorporated [125I]IdUrd. The SCC8 tumor was inoculated into castrated male mice and the mice were treated with 400 µg/day of TP. Two weeks after tumor inoculation, [125I]IdUrd was injected and the mice were divided into 5 groups. The mice in one group were killed 4 h after injection of [125I]IdUrd. The mice of one group did not receive any further injection of TP (TP 0), while those in the other three groups received daily injection of 100, 200, and 400 µg/day of TP, respectively (TP 100, TP 200, and TP 400). Mice with or without TP injections were killed on days 4, 9, and 13 after injection of [125I]IdUrd. Points, means ± SE (bars) of values in 5-6 tumors. a, significant difference from the values for tumors in mice killed 4 h after injection of [125I]IdUrd (P < 0.05 by Student's t test). b, significant difference from the values for tumors in mice without TP injections (P < 0.05 by Student's t test).

Host cells, such as fibroblasts, endothelial cells, and blood cells, have only low levels of androgen receptor. Thus, from our results it seemed likely that the amount of androgen receptor in SCC8 tumor cells was not less than that in SC115 tumor cells. Consistent with this conclusion, cells from the SCC8 tumor were found to have more androgen receptor than those from the SC115 tumor in vitro.

When [3H]testosterone was injected into tumor-bearing mice, the total amount of the most potent androgens (testosterone and 5α-dihydrotestosterone) retained in SCC8 tumors was 44% of that in SC115 tumors. However, the amount of steroids extracted from SCC8 tumors was 66% of that extracted from SC115 tumors. The difference between these two values suggests that testosterone is more rapidly metabolized to less androgenic steroids in SCC8 tumors than in SC115 tumors. In fact, the amount of less androgenic steroids was found to be much more in SCC8 tumors than in SC115 tumors. Furthermore, cells from SCC8 tumors metabolized testosterone to less androgenic steroids more rapidly than cells from SC115 tumors in vitro. Thus, testosterone is probably metabolized to less androgenic steroids more rapidly in SCC8 tumor cells than in SC115 tumor cells and this difference may partly account for the different sensitivities of these two tumors to androgen. However, the increased metabolism of testosterone cannot alone explain the decreased sensitivity of SCC8 tumors to androgen; because increased metabolism of testosterone could be compensated for by injection of TP at high doses, it could not explain why the maximal growth rate of SCC8 tumors was less than that of SC115 tumors.

The retention of 125I radioactivity in whole SCC8 tumors decreased with or without TP injections, suggesting that some SCC8 tumor cells labeled with 125I died, regardless of TP injections. Massive cell death is recognized morphologically as necrosis and scattered cell death as apoptosis (11). Spontaneous apoptosis or necrosis has been observed even during growth of tumors of various types (11, 39). We observed apoptosis and necrosis in the outer and inner portions of the tumors, respectively. Thus, the decrease in retention of 125I radioactivity seemed to result from cell death of these two types.

TP injections reduced the loss of 125I radioactivity on day 13 after the start of treatments with various doses of TP, suggesting that TP reduced cell death. However, no significant difference was found in the apoptotic indices of tumors in mice with and
without TP treatment. Thus, it seems likely that with depletion of androgen, the SCC8 tumor cells die mainly by necrosis and that androgen diminishes necrosis. Epithelial cells of the seminal vesicles and prostate and of the uterine die after depletion of androgen and estrogen, respectively, and these epithelial cells die by apoptosis, not by necrosis (9–13). Thus, our results suggest that the mechanism of the protective effect of steroids against cell death is different in normal target tissues and in steroid-dependent tumors. The necrosis in the center of tumors is almost certainly the result of hypoxia and ischemia (11, 39). Moreover, it has been reported that solid tumors secrete an angiogenic factor(s) and that inhibition of angiogenesis causes regression of tumors (40–43). Therefore, it is conceivable that TP stimulates SCC8 cells to secrete an angiogenic factor(s) that prevents ischemia and hypoxia. This possibility requires investigation.

The retention of 125I radioactivity in tumors in mice with or without TP treatment at various doses was not significantly different until day 9 of treatment. However, the sizes of the tumors differed, depending on the dose of TP. Because TP increased the mitotic index dose dependently, the growth of tumors seemed to depend largely on its stimulation of cell proliferation as shown in estrogen-responsive MXT mammary tumors (44).

Because the SCC8 tumor is androgen dependent during all phases of its growth, it is useful for investigating the action of androgen on cell death. The present study suggests that androgen may decrease cell death.

REFERENCES

Androgen Dependency of a Tumor Produced by a Cell Line Derived from Androgen-responsive Shionogi Carcinoma 115

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