Effect of Serial Passage in Female Nude Athymic Mice on Androgen Dependency of Shionogi Carcinoma 115

Yukihiko Kitamura, Naomi Uchida, Isamu Hayata, Kenji Yamaguchi, Shigeru Okamoto, Norihiko Narita, and Keishi Matsumoto

Institute for Cancer Research and Departments of Pathology and Surgery, Medical School, Osaka University, Kita-ku, Osaka, 530 [Y. K., S. O., N. N., K. M.]; Shionogi Research Laboratory, Shionogi Co., Ltd., Fukushima-ku, Osaka, 553 [N. U., K. Y.]; and Division of Radiation Health, National Institute of Radiological Sciences, Chiba City, 260 [I. H.], Japan

ABSTRACT

When Shionogi carcinoma 115 (SC115, an undifferentiated medullary carcinoma showing a compact cell pattern and containing androgen receptor) was transplanted into male and female DS mice, it grew only in males. In contrast with this strict androgen dependency in DS hosts, SC115 tumors grew in both male and female nude athymic (BALB/c-nu/nu) mice. Although most of the tumors developing in female nude mice were composed of spindle-shaped cells and did not contain androgen receptor, about 5% of tumors in female nude mice retained morphological and biochemical characteristics of the original SC115 tumor. Such a tumor was serially transplanted in female nude mice. Although no significant changes were detectable in histological and chromosomal features and in androgen receptor values, the growth speed in female nude mice accelerated and became comparable to the growth speed of the original SC115 tumor in intact male DS mice. However, this subline of SC115 tumor showed a marked androgen dependency when reinoculated into intact male and female DS mice after 14 passages in female nude mice in spite of its relative androgen independence in nude hosts. Therefore, the present results seem to suggest that the immunological status of the hosts may affect the hormone dependency of tumors.

INTRODUCTION

Androgen-dependent mouse mammary carcinoma SC115 was established in 1964 by Minesita and Yamaguchi (17, 18). The original tumor arose spontaneously in a female DS mouse and grew equally well when transplanted into male and female DS mice. After passage in male DS mice for 19 generations, the tumor was found to be androgen dependent, by its failure to grow in female or castrated male DS mice and by its ability to grow in female or castrated male DS mice given androgen. We (16, 19, 27–29) and other investigators (2–6, 10, 11, 15, 24, 26) have shown that SC115 cells contain a specific AR system, which has binding properties similar to those of androgen target tissue. Since SC115 cells have been shown to retain their androgen responsiveness in cell culture (6, 8, 10, 11, 24, 26), it seems that androgen stimulation of the growth of SC115 cells is directly mediated by AR in SC115 cells.

In contrast to strict androgen dependency in DS hosts, SC115 tumor was able to develop in female and in castrated male nude athymic mice (12). Our recent experiments demonstrated that SC115 cells changed their morphological, biochemical, and biological characteristics within one passage in androgen-depleted nude athymic hosts (12). Although the original SC115 tumor with positive AR is undifferentiated medullary carcinoma showing a compact cell pattern, most tumors developing in androgen-depleted nude mice are composed of spindle-shaped cells and do not contain AR. Because the spindle cell tumors induce bone formation in the surrounding connective tissues, they are easily recognized by their hardness and therefore were designated as "hard" tumor. However, about 5% of tumors developing in androgen-depleted nude mice did not show such hardness and were designated as "soft" tumor due to their relative softness (12). Histological and biochemical examination of the soft tumors revealed that they had the same histological characteristics as did the original SC115 tumors and that they contained AR. In order to establish a subline of SC115 which lacked biologically defined androgen dependency in spite of the presence of intracellular AR, we carried out serial transplantation of the soft tumor in female nude mice. After 14 generations in female nude mice, we retransplanted this subline into male and female DS mice and found that the tumor could grow in male DS mice but not in females. Inasmuch as our findings suggest that androgen dependency of the tumor may be influenced by the immunological status of the host, we examined some characteristics of this subline and report here.

MATERIALS AND METHODS

Animals and Tumors. DS mice were raised in our laboratory. Nude athymic mice (BALB/c-nu/nu) were purchased from the Japanese Central Laboratory for Experimental Animals (Tokyo, Japan). Nude mice were kept within a filtered-air laminar flow enclosure. Both DS and nude mice were used at 2 to 4 months of age. When castrated animals were used, the operation was carried out at least 1 week in advance. The tumor seeds of SC115 for transplantation were obtained from the 251st generation. The transplantation of tumor was done according to the method described in a previous paper (17).

Androgen Injection. TP was suspended in 0.05 ml of steroid solution (0.9% NaCl, 0.4% polysorbate 80, 0.5% carboxymethylcellulose, and 0.9% benzyl alcohol) and injected s.c.

Determination of Tumor Growth. Appearance of tumors was observed twice a week for 2 months. After tumor development, mice were kept until death or sacrifice. The length and width of each tumor were determined on every seventh day.
and the mean of the length and width was used as an index of tumor size. In one experiment, mice were killed at the 14th day after tumor grafting; tumors were removed and weighed. The weight of the tumor was used as an index of tumor growth.

**Assay for Cytoesol AR.** The assay method was described previously (12, 13). AR was determined by the dextran-coated charcoal assay using [3H]-5α-dihydrotestosterone (58 Ci/mmole) as radioactive steroid. The number of binding sites and the dissociation constant were calculated according to the procedure of Scatchard (23).

**History.** Tumors were fixed in 10% buffered formalin (pH 7.2) and embedded in paraffin; sections were stained with hematoxylin and eosin.

**Chromosome Analysis.** The method was described previously (12). The number of chromosomes was determined by Q-banded slides; karyotypes were made according to the standard karyotype of the mouse recommended by the Committee on Standardized Genetic Nomenclature for Mice (7).

**Assay for Serum Androgens.** In the testosterone radioimmunoassay, the antibody, raised against testosterone-3-carboxymethylxime-bovine serum albumin, was supplied by Dr. H. Imura, Kyoto University Medical School, and the radioactive steroid used was [3H]testosterone (40 Ci/mmole). This antibody cross-reacts with 5α-reduced androstans [5α-dihydrotestosterone (65%), 5α-androstane-3α,17β-diol (17%), and 5α-androstane-3β,17β-diol (7%)] but not with 17β-estradiol, progesterone, cortisol, or cortisone. Since serum testosterone was extracted with distilled methylene chloride and measured by the radioimmunoassay without chromatographic purification, serum testosterone levels shown in Table 4 may include some of 5α-reduced C₁₉ steroids. The intra- and interassay coefficients of variation obtained from 10 assays using mouse serum were 8.2 and 9.9%, respectively.

In the 4-androstene-3,17-dione radioimmunoassay, the antibody, raised against 4-androstene-3,17-dione-3-carboxymethylxime-bovine serum albumin, was supplied by Dr. T. Inaba, University of Osaka Prefecture. The radioactive steroid used was [3H]4-androstene-3,17-dione (36 Ci/mmole). This antibody slightly cross-reacts with androsterone (6%), dehydroepiandrosterone (3%), and testosterone (2%) but not (less than 0.2%) with 17β-estradiol, estrone, progesterone, cortisol, corticosterone, or pregnenolone. The intra- and interassay coefficients of variation obtained from 10 assays using mouse serum were 8.2 and 9.9%, respectively. The level of 4-androstene-3,17-dione in adult male rat serum was found to be 0.87 ± 0.09 (S.E.) ng/ml which was similar to the level reported by Resko et al. (21).

**RESULTS**

SC115 tumor was grafted to 70 female nude mice. Within 2 months after transplantation, 63 hard and 3 soft tumors appeared. One of the soft tumors was used as the seed of the next generation and was grafted to 10 female nude mice. Although the hard tumors did not appear in the second and third generations, 8 of 10 tumors in the fourth generation were hard. However, hard tumors never appeared after the fourth generation (Chart 1). The survival time of the female nude hosts after the tumor transplantation began to decrease from the fourth generation and dropped to the same level as the survival time of intact male DS hosts grafted with the original SC115 tumor (Chart 1). Therefore, the soft tumor was maintained in female nude mice as a subline of SC115 tumor.

Histologically, the subline remained a medullary carcinoma showing a compact cell pattern after 14 serial passages in female nude mice and could not be discriminated from the original SC115 tumor. Although the modal number of chromosomes increased from 40 in the original SC115 tumor to 41 in the subline during the serial passages in nude mice, detailed examination by the Q-banding method revealed that the increase was attributable to the addition of the No. 15 chromosome. The No. 15 chromosome showed trisomy in the original SC115 tumor (12), but tetrasomy in the soft tumor maintained in female nude mice.

After 14 serial passages in female nude mice, the soft tumor was transplanted to female and male nude mice and female and male DS mice. Transplantability to female DS mice was significantly lower than that to male DS mice in spite of the fact that the tumor grew in 99% of both female and male nude mice (Table 1). Growth speed of the tumors in female nude mice was moderately slower than that in male nude mice and was slightly slower than that in male DS mice (Chart 2).

The AR level and dissociation constant of the subline did not change significantly from those of the original SC115 tumors maintained in intact male DS mice (Table 2). The dissociation constant of the subline and the original SC115 tumor was 3 to 10 × 10⁻¹⁰ M, indicative of high-affinity binding. As retention of tightly binding AR's and maintenance of specificity for the male DS hosts suggested that androgen responsiveness was retained by the subline, the next experiment was carried out to examine this point. Castrated male DS mice were grafted with the subline tumors, and some of the recipients received consecutive injections of TP. The tumor weights were measured at the 14th day after the transplantation. The weight of the seminal vesicle was also measured to refer the effect of TP injections. The tumor growth was significantly accelerated in castrated male DS mice receiving TP injections (Table 3). The higher dose of TP (400 μg/day) was more effective than was the moderate dose (100 μg/day).

**Chart 1.** Transplantability of each generation of soft tumor in female nude mice. Seventy mice were used for the first generation (primary transplantation from the original SC115 tumor maintained in male DS mice). After the second generation, 10 mice were used for each generation. Transplantability of the original SC115 tumor in male and female DS mice during the present study is shown for comparison. Mean survival times of female nude mice for each generation and survival times of male and female DS mice transplanted with the original SC115 tumor are also shown. Bars, S.E.
Table 1

Transplantability into various hosts of subline of SC115 tumor maintained in female nude mice

<table>
<thead>
<tr>
<th>Generation</th>
<th>Female nude</th>
<th>Male nude</th>
<th>Female DS</th>
<th>Male DS</th>
<th>(p^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>10/10</td>
<td>10/10</td>
<td>1/6</td>
<td>6/6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>16</td>
<td>10/10</td>
<td>10/10</td>
<td>1/6</td>
<td>5/6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>17</td>
<td>9/10</td>
<td>10/10</td>
<td>1/7</td>
<td>4/5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>18</td>
<td>10/10</td>
<td>10/10</td>
<td>3/9</td>
<td>10/12</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>19</td>
<td>10/10</td>
<td>10/10</td>
<td>3/8</td>
<td>4/7</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>20</td>
<td>10/10</td>
<td>8/9</td>
<td>0/8</td>
<td>8/8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>21</td>
<td>10/10</td>
<td>10/10</td>
<td>3/12</td>
<td>10/10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total</td>
<td>68/70 (99)c</td>
<td>68/69 (99)</td>
<td>12/49 (24)</td>
<td>47/54 (87)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

a Lethal growth of tumor in 60 days after transplantation.
b Transplantability was compared between female and male DS mice by \(\chi^2\) test.
c Numbers in parentheses, percentage.

Table 2

AR levels of subline of SC115 tumor maintained in female nude mice and transplanted to various hosts

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Host</th>
<th>AR level (fmol/mg cytosol protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subline</td>
<td>Female nude</td>
<td>22.2 ± 1.7a,b (6)b</td>
</tr>
<tr>
<td></td>
<td>Male nude</td>
<td>34.7 ± 2.4b (6)</td>
</tr>
<tr>
<td></td>
<td>Male DS</td>
<td>21.6 ± 2.9b (3)</td>
</tr>
<tr>
<td>Original SC115</td>
<td>Male DS</td>
<td>31.1 ± 4.3 (6)</td>
</tr>
</tbody>
</table>

a Mean ± S.E.
b \(p > 0.1\) when compared to the value of the original SC115 tumor in male DS mice by t test.
c Numbers in parentheses, number of mice.

Table 3

Androgen responsiveness of the subline tumor grafted to castrated male DS mice

<table>
<thead>
<tr>
<th>Dose of TP ((\mu g/\text{mouse/day}))</th>
<th>No. of mice</th>
<th>Wt of tumor (g)</th>
<th>(p^a)</th>
<th>Wt of seminal vesicle (mg)</th>
<th>(p^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>0.6 ± 0.3</td>
<td>&lt;0.001</td>
<td>9 ± 1.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>100</td>
<td>10</td>
<td>2.5 ± 0.3</td>
<td>&lt;0.05</td>
<td>43 ± 2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>400</td>
<td>10</td>
<td>4.2 ± 0.4</td>
<td>&lt;0.001</td>
<td>58 ± 2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

a Adjacent values were compared by t test.
b Mean ± S.E.

Since there is a possibility that testosterone concentration in female nude mice might be high enough to explain the growth of the AR-positive soft tumors in the latter mice, we determined the serum testosterone levels in nude and DS mice of both sexes. However, there was no significant difference in testosterone levels between female nude and female DS mice, and both levels were much lower than those in male nude and male DS mice (Table 4). Although androgens produced by the adrenal cortex contribute insignificantly to the total androgen load in normal mice, it is possible that the adrenals of nude mice might produce a very large amount of weaker androgens which could satisfy the androgen requirement of the AR-positive subline tumor. However, there was no significant difference in 4-androstene-3,17-dione levels between female nude and female DS mice (Table 4). Furthermore, the weight of the seminal vesicle in castrated male nude mice was similar to that in castrated male DS mice but much smaller than the values in intact male nude and DS mice, showing that the adrenal cortex of nude mice does not secrete such a large quantity of adrenal androgens (Table 4).

DISCUSSION

Most of tumors appearing in female nude mice after transplantation of androgen-dependent medullary SC115 tumors were composed of spindle-shaped cells. They lack AR and grew in female as well as male DS mice when reinoculated (12). However, about 5% of tumors appearing in female nude mice had the same histological features as those of the original SC115 tumor and were easily recognized by their relative softness. We established a subline of SC115 tumor by serial transplantation of the soft tumor to female nude mice. Although they retained the original histological and chromosomal characteristics and contained cytosol AR even after 14 serial passages in female nude mice, the growth speed in female nude hosts accelerated and became comparable to the growth speed of the original SC115 tumors in intact male DS hosts. In spite of its relative androgen independency in nude hosts, this subline of SC115 tumor showed a marked androgen dependency when retransplanted into male and female DS hosts. Therefore, the subline does not seem to be identical to the autonomous subline of SC115 tumor described by Bruchovsky and Rennie (4) which had cytosol AR but showed a postreceptor defect for androgen action.

Serum testosterone concentration and production of adrenal androgens including 4-androstene-3,17-dione did not give a reasonable explanation for the growth of AR-positive cells in the latter. Although it also seems possible that the observed results might be caused by endocrine differences other than androgen levels in nude and DS mice, the apparently intact function (20) and morphology (22) of the thyroid, adrenal
cortex, and pituitary in nude mice were reported. Moreover, our recent results have shown that growth of SC115 tumors in DS mice is stimulated only by androgens but not by estrogens, progestins, glucocorticoids, prolactin, or other pituitary hormones (14). Thus, the difference between nude and DS hosts in androgen dependency may be attributed to the difference in immunological status of hosts defined by the absence or presence of the thymus. Although the absence of the thymus does not necessarily predispose the development of spontaneous or chemically induced tumors, there have been many experiments demonstrating the effect of T-lymphocytes on the growth of tumors (25). The present results suggest that T-lymphocytes may also affect the hormone dependency of the tumor. In the previous report, we showed that removal of androgen did not suppress the growth of SC115 tumors in DS hosts after the diameter of tumors exceeded 28 mm (13). The unresponsiveness of the very large tumors to androgen removal might be partly attributed to the immunodepression of the hosts by the tumor.

Drago et al. (9) reported that the hormone responsiveness of rat prostate cancers was preserved after serial passages in nude mice. On the other hand, Bogden et al. (1) described changes in biological and chemotherapy response characteristics of rat mammary cancers in nude mice. In fact, one of 2 mammary cancers used by Bogden et al. did not grow when it was reimplanted into previously syngeneic rats after 20 serial passages in nude mice. Our present results show both aspects proposed by these 2 groups of researchers. The strict androgen dependency of the SC115 tumor observed in DS mice was much reduced by serial transplantation into female nude mice. On the other hand, the strict androgen dependency reappeared when the tumor maintained in female nude mice was reimplanted to DS hosts. Therefore, the present results suggest that caution is indispensable in interpreting the results obtained in tumor allo- and xenograft-nude mice experimental systems.

ACKNOWLEDGMENTS

We thank Dr. K. Takeda and Dr. H. Otsuka for supporting these studies.

REFERENCES


Table 4

| Serum androgen concentration and weight of seminal vesicle in various mice
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Serum concentration (ng/ml)</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Intact female nude</td>
</tr>
<tr>
<td>Castrated male nude[^e]</td>
</tr>
<tr>
<td>Intact male nude</td>
</tr>
<tr>
<td>Intact female DS</td>
</tr>
<tr>
<td>Castrated male DS[^e]</td>
</tr>
</tbody>
</table>

[^a] Mean ± S.E.
[^b] p < 0.05 when compared to the value of intact male mice of the same strain.
[^c] p > 0.05 when compared to the value of intact female DS mice.
[^d] Numbers in parentheses, number of mice.
[^e] Castrated 2 weeks before sacrifice.


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