Effect of Androgen Depletion on Growth and Androgen Dependency of Shionogi Carcinoma 115

Yukihiko Kitamura, Shigeru Okamoto, Naomi Uchida, Kenji Yamaguchi, and Keishi Matsumoto

Institute for Cancer Research, Osaka University Medical School, Kita-ku, Osaka, 530 [Y. K., S. O., K. M.], and Shionogi Research Laboratory, Shionogi Co., Ltd., Fukushima-ku, Osaka, 553 Japan [N. U., K. Y.]

ABSTRACT

Shionogi carcinoma 115 (SC115) tumor showed strict androgen dependency for initiation of growth. Inoculated SC115 cells showed evidence of necrosis within 5 days after implantation in the absence of androgen. After initiation of growth by androgen, the subsequent fate of SC115 tumors following androgen removal depended on the size of the tumor; small, medium, and large tumors showed complete, temporary, and no regression, respectively. In contrast with the partial and no androgen dependency of medium and large tumors in situ, tumor seeds taken from the medium and large tumors before androgen removal grew only in males when transplanted into male and female mice. On the other hand, tumor seeds taken from about one-half of the regrown tumors after androgen removal grew in both male and female hosts. One type of these androgen-independent tumors contained cytosol androgen receptor (AR) and consisted of the original SC115 cells (mediulary cancer) and spindle-shaped cells. Another type of independent tumor contained no cytosol AR and consisted only of spindle-shaped cells. These findings seem to show that the effect of androgen depletion on the growth of tumor mass, which consists of AR-positive and androgen-dependent cells, varies in different phases of tumor growth and that the androgen dependency of tumor cells can be changed after removal of androgen. The SC115 tumor seems to be a good model for elucidating hormone-dependent cancer growth and for establishing endocrine treatment in hormone-dependent cancers.

INTRODUCTION

Androgen-dependent mouse mammary carcinoma SC115 was established in 1964 by Minesita and Yamaguchi (18, 19). The original tumor arose spontaneously as an androgen-independent adenocarcinoma of mammary origin in a female DS mouse and grew equally well when transplanted into male and female mice. After passage in male DS mice for 19 generations, the tumor was found to be androgen dependent, defined by its failure to grow in either female or castrated male mice and by its ability to grow in female or castrated male mice given androgen. Cells derived from this tumor retain their androgen responsiveness in vivo and in cell culture (6, 8, 11, 12, 23, 24). We (15, 20, 25, 26) and other investigators (2-6, 8, 11, 12, 14, 23, 24) have reported that androgen action on the stimulation of SC115 tumor growth is mediated by a specific AR system in SC115 cells, which have binding properties similar to those of androgen target tissues.

SC115 cells inoculated into androgen-deprived mice usually do not grow. In contrast, our preliminary experiments showed that castration of males bearing relatively large SC115 tumors did not result in complete regression of the tumor. Most of these tumor-bearing mice died from the tumor after a temporary regression. A similar observation has been made by Desmond et al. (8). Because it is possible that the androgen dependency of the SC115 tumor varies in different phases of growth, the fate of SC115 tumors in an androgen-depleted state is shown in detail here.

MATERIALS AND METHODS

Animals and Tumors. DS mice, raised in our laboratory, 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 246th to the 258th generations. In each experiment intact male and female DS mice were grafted with the same tumor seeds to ensure androgen dependency of the tumor. Cumulative lethal takes during the experimental period were 133 of 155 (86%) in males and 5 of 130 (4%) in females. The method of tumor transplantation was described previously (18).

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.

Histology. Tumors were fixed in 10% buffered formalin (pH 7.2) and embedded in paraffin; sections were stained with hematoxylin and eosin. Serial sections were made to examine small samples.

Determination of Tumor Growth. Length and width of each tumor were measured on every fifth day, and the mean of the length and width was used as an index of tumor size. In one experiment proliferation of tumor cells was determined by measuring the incorporation of [125I]IdUrd into the whole tumor. Mice were given i.p. injections of 0.1 μmol of fluorodeoxyuridine to inhibit endogenous thymidylate synthesis (1). After 1 hr, 1 μCi of [125I]IdUrd was injected i.p. Mice were killed 3 hr after injection of [125I]IdUrd (1), and the whole tumor was removed and fixed in 10% buffered formalin (pH 7.2). Tumors were cut into 1-mm slices after overnight fixation. The 125I not incorporated into DNA was removed by soaking the sliced tumors in 10% formalin for at least 5 days with daily changes. The 125I retained in DNA...
was measured in an auto-well γ counter. The results were expressed as the mean tumor uptake of [125I]IdUrd (percentage of injected radioactivity).

Fate of Tumors. The fate of each tumor after removal of androgen was classified according to the following criteria: (a) complete regression, in which tumor size decreases after androgen removal and does not increase again in the following 90 days. The host animal does not die due to the tumor; (b) temporary regression, in which tumor size decreases after androgen removal and remains in the decreased size for at least the following 15 days. The tumor resumes growth until the host dies; (c) no regression, in which the tumor growth is not affected by androgen removal.

Assay of Cytosol AR. When noncastrated mice were used as hosts, the mice were castrated 18 hr before removal of the tumors. The tumors were excised and used immediately. All the following procedures were carried out at 4°C. The tumors were homogenized in 8 volumes of 10 mM Tris-HCl-1.5 mM EDTA-0.5 mM dithiothreitol, pH 7.4 (Tris buffer), with a Polytron homogenizer. The homogenates were centrifuged at 105,000 × g for 60 min to obtain the cytosol fractions. Two hundred μl of cytosol fractions (3 to 4 mg of protein per ml) prepared in the Tris buffer were incubated with increasing quantities of [3H]dihydrotestosterone (17β-hydroxy-5α-androstan-3-one), 58 Ci/mmol (0.1 to 3.2 pmol/ml), for 16 hr. Control cytosols were preincubated with 300 pmol of nonradioactive dihydrotestosterone per ml 20 min prior to the addition of the [3H]dihydrotestosterone. The total volume of the reaction mixture was 0.4 ml. After incubation, 0.4 ml of dextran-coated charcoal suspension (Norit A, 0.5 g/100 ml, and dextran, 0.005 g/100 ml, in 0.01 M Tris-HCl, pH 8.0) was added to each tube, followed by incubation for 30 min with intermittent vortexing. The tubes were then centrifuged at 1500 × g for 10 min, and aliquots of supernatants were taken for estimating radioactivity. The results were analyzed according to the procedure of Scatchard (22). A tumor was considered AR negative if it contained less than 5 fmol AR per mg cytosol protein. Protein was quantitated by the method of Lowry et al. (13).

RESULTS

Complete Dependency on Androgen for Initiation of Tumor Growth. The SC115 tumors were implanted in castrated male mice, and TP (100 μg/mouse) was injected daily for 60 days starting from various days after the tumors were grafted. The ratio of lethal growth during the TP injection and the mean survival time in tumor-bearing mice were determined. All castrated mice, which received TP for 60 days starting from the day of tumor implantation, died due to the tumor. Although most of the mice (84%) given TP from 1 day after tumor grafting died as well, the mean survival time was significantly longer than that of mice given TP from the day of tumor grafting (Table 1). In contrast with the high mortality of mice given TP for 60 days starting from 0 or 1 day after implantation of SC115 tumor, little tumor growth and few deaths were found in the castrated mice that were given TP from 5 to 21 days after implantation of tumors (Table 1). The lack of TP for 5 days after grafting had definite effects on tumor growth, since TP injection for 60 days thereafter had very little or no stimulative effect on tumor growth. Histological examination of serial sections of SC115 tumors at 5 days after implantation showed that SC115 cells were actively growing in the presence of androgen (daily injection of 100 μg TP per mouse), whereas the tumor cells showed evidence of necrosis in the absence of androgen.

The daily dose of TP was changed in the next experiment. The growth of SC115 tumors was accelerated, and the mean survival time was significantly shortened in castrated mice given injections of larger amounts of TP (Table 2). The rate of lethal growth in 60 days was 100% in castrated mice given 100 or 400 μg of TP starting from the day of implantation, but was only 40% in castrated mice given 25 μg of TP starting from the day of implantation (Table 2). The results shown in Table 2 also support the concept that androgen is required for initiation of SC115 tumor growth.

The failure of tumor maintenance by injection of low
doses of TP in castrated male mice is shown in Table 3. The SC115 tumors were implanted in castrated mice, and the mice were kept for 4 or 9 days in an androgen-deficient condition by daily injection of 10 μg TP per mouse starting from the day of implantation. The low androgen condition for 4 or 9 days after grafting had definite effects on tumor growth, since daily injection of 100 μg TP per mouse for 60 days thereafter had very little stimulative effect on tumor growth. Ratios of lethal growth were similarly low in castrated mice maintained for 4 or 9 days in the absence or deficiency of androgen (Table 3).

**Androgen Depletion after Initiation of Tumor Growth.** Seventy-nine castrated male mice were grafted with SC115 tumors and given injections of TP (daily dose of 100 μg/mouse) from the day of grafting. Then the TP injection was stopped from various days after implantation of the tumor. As shown in Chart 1 and Table 4, the subsequent fate of SC115 tumor depended on the size of the tumor at the time TP injection was stopped. Most of the small tumors (less than 14 mm in diameter) regressed completely. Tumors of medium size (14 to 28 mm in diameter) showed a temporary regression, then they resumed growth, and the host animals died due to the tumor. However, the mean survival time of the hosts was significantly lengthened when the tumor showed temporary regression (Table 4). Large tumors (more than 28 mm in diameter) did not show any regression.

**Characterization of Tumor That Showed Temporary Regression.** Since 6 of 10 tumors showed temporary regression when TP injection was stopped from the 20th day after implantation (Chart 1), the character of SC115 tumors in and after the temporary regression was studied under the previously described experimental conditions.

DNA synthesis was evaluated by assaying the incorporation of [125I]dUlrd into the whole tumor. The [125I]dUlrd uptake increased progressively in mice given TP injections continuously (daily dose of 100 μg/mouse), whereas very little [125I]dUlrd uptake was found in noninjected control animals (Chart 2). After TP injection was stopped on the 20th day, the [125I]dUlrd uptake into the whole tumor did not decrease significantly despite the fact that the size of the tumor did not increase in the following 15 days (Chart 2).

**Table 3**

<table>
<thead>
<tr>
<th>Group</th>
<th>TP injection (days after grafting)</th>
<th>Ratio of lethal growtha</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None 0–60</td>
<td>10/10</td>
</tr>
<tr>
<td>2</td>
<td>None 0–60</td>
<td>10/10</td>
</tr>
<tr>
<td>3</td>
<td>None 5–65</td>
<td>1/8</td>
</tr>
<tr>
<td>4</td>
<td>None 5–65</td>
<td>3/9</td>
</tr>
<tr>
<td>5</td>
<td>None 10–70</td>
<td>1/8</td>
</tr>
<tr>
<td>6</td>
<td>None 10–70</td>
<td>2/10</td>
</tr>
</tbody>
</table>

a In Groups 1 and 3 to 6, lethal growth in 60 days after start of 100 μg TP injection. In Group 2, lethal growth in 60 days after start of 10 μg TP injection.

The number of binding sites for [3H]dihydrotestosterone in the cytosol fraction from temporarily regressing or regrowing tumors was determined on various days after removal of androgen. Positive dihydrotestosterone binding (more than 5 fmol/mg cytosol protein, with high affinity) was demonstrated in 27 of 28 tumors 5 to 91 days after TP injection was stopped (i.e., 25 to 111 days after transplantation). The AR values of 11 of these 28 tumors are given in Table 5.

SC115 tumors that resumed growth after temporary regression were transplanted into intact male and female DS mice. Tumor seeds from 6 of the 11 regrowing tumors...
actually grew only in the male hosts (Table 5, Tumors 1 to 4, 7 and 8). Positive androgen binding with high affinity was detectable in all these biologically androgen-dependent tumors (Table 5). Tumor seeds from the other 5 regrowing tumors grew in both male and female hosts (Table 5, Tumors 5, 6, and 9 to 11). One of these 5 tumors contained no cytosol AR (Table 5, Tumor 11). On the other hand, positive androgen binding was demonstrated in the other 4 tumors in spite of their biological independency on androgen (Table 5, Tumors 5, 6, 9, and 10). When receptor content of tumors transplanted from the latter 4 tumors was evaluated, positive androgen binding was detected not only in tumors in male mice but also in those in female mice (Table 5). The dissociation constants (K_d) for dihydrotestosterone of these AR-positive but androgen-independent tumors in the first and second generations were estimated to be 3 to 10 × 10^{-10} M, indicative of high-affinity binding. Similar K_d's were found in all AR-positive tumors examined in this study.

Histological examination of regrowing original tumors that showed positive androgen binding (Table 5, Tumors 1 to 10) revealed that SC115 cells (i.e., undifferentiated medullary cancer) were intermingled with clusters of spindle-shaped cells (Fig. 1). On the other hand, the tumor without androgen binding (Table 5, Tumor 11) consisted only of these spindle-shaped cells. The same histological features and the defect of receptor production of the latter tumor (Tumor 11) were observed in the next generation as well (Table 5). The spindle-shaped cells were not found in SC115 tumors before removal of androgen; small, medium, and large SC115 tumors consisted only of medullary cancer cells, which contained AR and, respectively, showed complete, temporary, and no regression after removal of androgen (Chart 1).

**DISCUSSION**

The findings shown in Tables 1 to 3 indicate that inoculated SC115 cells die within 5 days after implantation in the absence of androgen. In cell culture SC115 cells did not grow but remained viable after 8 days (23) or grew slowly (12) in the absence of androgens, and they could be stimulated to grow more rapidly by addition of androgens. The SC115 cells inoculated in mice exhibit a greater sensitivity to androgens (Tables 1 to 3) than do cells in culture. The explanation for this discrepancy seems to reside in the simpler pattern of growth control in culture as compared with that of the animal as a whole. In the animal, additional constraints exist such as immunological response of the host, the presence of multiple hormones, and the much more complex interactions between cells and available blood supply. These factors influencing the tumor growth *in vivo* are now under investigation.

### Table 5

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Days after TP injection was stopped</th>
<th>AR value of original tumor (fmol/mg cytosol protein)</th>
<th>Ratio of lethal growth</th>
<th>Mean AR value of transplanted tumor (fmol/mg cytosol protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>1</td>
<td>22</td>
<td>44</td>
<td>10/10</td>
<td>0/10</td>
</tr>
<tr>
<td>2</td>
<td>22</td>
<td>31</td>
<td>8/10</td>
<td>0/10</td>
</tr>
<tr>
<td>3</td>
<td>22</td>
<td>27</td>
<td>10/10</td>
<td>1/10</td>
</tr>
<tr>
<td>4</td>
<td>22</td>
<td>35</td>
<td>10/10</td>
<td>1/10</td>
</tr>
<tr>
<td>5</td>
<td>22</td>
<td>25</td>
<td>9/10</td>
<td>5/10</td>
</tr>
<tr>
<td>6</td>
<td>22</td>
<td>34</td>
<td>10/10</td>
<td>8/10</td>
</tr>
<tr>
<td>7</td>
<td>29</td>
<td>43</td>
<td>10/10</td>
<td>0/10</td>
</tr>
<tr>
<td>8</td>
<td>29</td>
<td>54</td>
<td>10/10</td>
<td>3/10</td>
</tr>
<tr>
<td>9</td>
<td>29</td>
<td>57</td>
<td>10/10</td>
<td>7/10</td>
</tr>
<tr>
<td>10</td>
<td>91</td>
<td>39</td>
<td>10/10</td>
<td>8/10</td>
</tr>
<tr>
<td>11</td>
<td>91</td>
<td>&lt;5</td>
<td>9/10</td>
<td>8/10</td>
</tr>
</tbody>
</table>

* TP injection was stopped from the 20th day after implantation of SC115 tumors.

* Lethal growth in 60 days.

* Numbers in parentheses, number of mice.

* NE, not examined.
The SC115 tumors regressed completely when TP injection was stopped before the diameter of the tumor reached 14 mm (Chart 1; Table 4). This result is consistent with the results discussed in the preceding paragraph that androgen is mandatory for growth initiation of SC115 tumors. In contrast with the complete regression found after the removal of androgen from small SC115 tumors, the SC115 tumors showed only temporary or no regression after androgen removal when they had reached a considerable size [more than 14 mm (Chart 1; Table 4)]. However, each tumor cell or cell group in the tumor masses that had become large seemed to retain androgen responsiveness before androgen removal, since our previous experiments have shown that tumor seeds from these medium and large SC115 tumors grow when transplanted into males but not females. In fact, the androgen-dependent character of SC115 cells has been maintained in our laboratory for more than 10 years by transplantation into male mice of tumor seeds taken from large tumors (approximately 30 mm in diameter). These medium and large tumors before androgen removal consist only of medullary cancer cells and contain cytosol and nuclear AR (15, 20, 25, 26). The present findings show that the effect of androgen depletion on the growth of SC115 tumor mass, which consists of AR-positive and androgen-dependent cells, varies in different phases of tumor growth. Perhaps the most interesting observations in this study are that very large tumors will not regress upon androgen withdrawal but that they can be transplanted successfully only to male mice (not females) and have morphology and AR levels similar to the smaller tumors, which regress upon androgen withdrawal. It is strange that the large tumor masses that consist of AR-positive and androgen-dependent tumor cells lose their responsiveness to androgen. This should be clarified in detail by future studies in order to elucidate hormone-dependent cancer growth. In human breast cancers regressions following endocrine therapy have been found in 30% of metastatic cancers (7) and in 50 to 60% of estrogen receptor-positive cancers (16). Like SC115 tumors of medium size in mice, regression of metastatic human breast cancers following endocrine therapy is for the most part temporary. Our findings suggest that endocrine therapy might be more effective for controlling the growth of human breast cancers in the early stages of development. In human breast cancers, approximately 30% of estrogen receptor-positive and progesterone receptor-positive cancers do not respond to endocrine therapy (17). The present findings suggest that some of these tumor masses may consist of estrogen-responsive cancer cells as do SC115 tumors of large size [more than 20 mm (Chart 1)].

The fact that about one-half of the SC115 tumors that regrew after androgen withdrawal could be transplanted into female mice indicates that the biological features of some SC115 tumors seem to change in the absence of androgen from androgen dependency to androgen independency following the temporary regression (Table 5). The histological features of the SC115 tumors also changed after androgen removal, i.e., the appearance of spindle-shaped cells following the temporary regression (Fig. 1). Since the androgen-independent tumor without androgen binding (Table 5, Tumor 11) consisted only of spindle-shaped cells and the androgen-independent tumors with androgen binding (Table 5, Tumors 5, 6, 9, and 10) consisted of the original SC115 tumor cells (medullary cancer) and the spindle-shaped cells (Fig. 1), the development of the spindle-shaped cells without AR might change the biological character of SC115 tumors from androgen dependent to androgen independent. In Tumors 5, 6, 9, and 10, these spindle-shaped cells did not become the predominant cell type (less than medullary cancer cells) either in the original tumors or in tumors transplanted later in male and female mice. Perhaps a very interesting observation in this study may be that some of the regrown tumors following androgen withdrawal are successfully transplanted to both male and
female mice but contain normal levels of AR. Since the emergence of clusters of the spindle-shaped cells without AR is responsible for this discrepancy, the origin and nature of the spindle-shaped cells should be clarified in future studies. In human prostate cancers approximately 80% of metastatic cancers are initially clinically responsive to either surgical castration or medical pituitary-gonadal suppression, but eventually regrowth of tumor tissue occurs (9). In human breast cancers the response duration for endocrine ablation is at most only 6 to 12 months (10), which indicates that biologically significant but very small amounts of estrogen is uncertain origin may be stimulating tumor growth or that the cancers have become hormone independent. Our present results seem to suggest that hormone dependency of tumor cells can be changed in the absence of hormones. Although regrowing breast tumors after temporary regression by endocrine therapy have been reported to retain estrogen receptors (21), these findings do not rule out the estrogen-independent cancer cells without estrogen receptors appearing together with the estrogen-dependent cancer cells with estrogen receptors (see Table 5, Tumors 5, 6, 9, and 10).

Recently, Bruchovsky and Rennie (4) reported that cytosol AR concentration, androgen uptake into the nucleus, and displaceable nuclear binding provided sufficient information to predict dependence or independence for 100% of 11 variant lines of SC115 tumors, although hormone dependence could not be completely predicted by cytosol AR concentration alone. It seems that extensive studies on receptor systems for various hormones are required in order to obtain ideal markers for selecting endocrine-responsive tumors. However, the present findings suggest that the satisfactory selection of endocrine-responsive tumors may not be achieved by studies on the hormone-receptor system alone. The SC115 tumor seems to be a good model for elucidating hormone-dependent cancer growth and for establishing endocrine treatment in hormone-dependent cancers.

ACKNOWLEDGMENTS

We thank Dr. K. Takeda and Dr. H. Otsuka for supporting these studies and S. Yagi for correcting our English.

REFERENCES


Effect of Androgen Depletion on Growth and Androgen Dependency of Shionogi Carcinoma 115

Yukihiko Kitamura, Shigeru Okamoto, Naomi Uchida, et al.