Hormone Dependency of a Serially Transplantable Human Prostatic Cancer (HONDA) in Nude Mice

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ABSTRACT

Human prostatic cancer (HONDA) serially transplanted in nude mice grew well in male mice but not at all in untreated female mice or in castrated male mice. Progressive growth in female mice was obtained by i.m. administration of 1 mg of testosterone twice a week. Estradiol inhibited the growth of the tumor in male mice to some extent; however, some growth was observed.

The tumor in untreated male mice retained the histological features of poorly differentiated adenocarcinoma. Tumors in castrated male mice showed reduction in size of tumor cell nests with relative overgrowth of stroma. The tumor in androgenized female mice consisted of columnar epithelial cells with large nuclei and more abundant cytoplasms and a large glandular lumen, showing histology of moderately differentiated adenocarcinoma.

High levels of human prostatic acid phosphatase (PAP) were detected in sera from untreated male mice. Testosterone markedly increased the content of serum PAP of androgenized female mice. Estradiol reduced the levels of PAP in sera from untreated male mice regardless of the tumor weight.

High-affinity androgen receptors were present in cytosol and in nuclear extract of the tumor in untreated male mice. No measurable amount of progesterone or estrogen receptors was present in cytosol from untreated male mice.

INTRODUCTION

Androgens appear to be necessary for the initiation of human prostatic cancer and the maintenance of tumor growth (1–3). About 75% of all prostate cancers respond to endocrine therapy for varying periods of time in the clinical course of the disease (4). Orchiectomy, administration of estrogens, progestins, or antiandrogenic compounds have been accepted as a form of treatment (5).

Contents of the androgen receptor have provided an explanation of hormone sensitivity of this cancer (6, 7), which suggests that androgens might be directly involved in regulation of growth of the tumor cells. However, mechanisms of the hormone-dependent growth of the cancer cells have not been yet elucidated. The investigation may only be accomplished properly by the use of a model system originated from human prostate. Basic research on the androgen-dependent cellular process in prostatic cancer cells provides evidence to understand one aspect of this cancer from its various biological features, despite the heterogeneity of the cancer which has been proposed (8).

Despite successful establishment of cell lines from human prostatic cancer (9–11) and serial passages of the cancer in athymic nude mice (12–14), no sufficient explanation of hormone dependency of human prostatic cancer cells has been noted. We previously reported briefly the establishment and properties of serially transplantable human prostatic cancer in nude mice (15).

In this study we report the effects of hormonal manipulation on tumor growth, induction of PAP, histology, and assays of the androgen, estrogen, and progesterone receptors to evaluate the usefulness of this model as a hormone-dependent human prostatic cancer.

MATERIALS AND METHODS

Chemicals. [17α-methyl-3H]R1881 (87 Ci/mmol), unlabeled R1881, [2,4,6,7-3H]estradiol (91 mCi/mmol), [17α-methyl-3H]R5020 (77.1 mCi/mmol), unlabeled R5020, and Aquasol-2 scintillation fluid were obtained from New England Nuclear (Boston, MA). 14C-methylated BSA (60 μCi/mg) was obtained from the Radiochemical Centre (Buckinghamshire, England). Testosterone, DHT, 17β-estradiol, progesterone, dexamethasone, DES, dithiothreitol, TA, and activated charcoal were all obtained from Sigma Chemical Co. (St. Louis, MO). Dextran (M, 80,000) was from Nakarai Chemical Ltd. (Kyoto, Japan). Testosterone propionate and estradiol dipropionate were from Teikoku Hormone Co. (Tokyo, Japan), and PAP radioimmunoassay kit was purchased from EIKEN ICL Co. (Tokyo, Japan).

Tumor. The tumor from metastatic carcinoma of the prostate was successfully transplanted to male nude mice in October 1977, and the tumor was designated HONDA (15). The tumor has been serially transplanted in our laboratory without failure, and it is now in the 25th passage. Serial transfer of the tumor was performed by transplanting several fragments of tumor (about 1 to 2 mm in diameter) into the right flank of the nude mice. Transplantation for the experiments was performed by the same method in male and female mice.

Nude Mice. Male and female nude mice, 7 to 8 weeks old, with a genetic background of BALB/c, were maintained in our laboratory under pathogen-limited conditions, and all of the treatments were performed under the same conditions.

Growth and Histology. The transplanted tumor was measured externally with calipers once/week. Tumor weight was calculated using the formula

\[ \text{Tumor weight (mg)} = \frac{W^2 \times L}{2} \]

where W is the width of the tumor in mm and L is the length in mm (16). Tumors produced by HONDA were removed, fixed in 10% phosphate-buffered formaldehyde solution, embedded in paraffin, and stained with hematoxylin and eosin.

Hormonal Manipulation. Testosterone propionate (1 mg in 50 μl of sesame oil) was injected i.m. into female mice twice a week for 8 weeks.
Estradiol dipropionate (100 µg in 50 µl of sesame oil) was injected i.m. into male mice once/week for 8 weeks. These hormones were all injected immediately after inoculation of the tumor. Bilateral orchietomy was performed via the scrotal route under ether anesthesia 4 weeks after tumor transplantation.

Prostatic Acid Phosphatase. PAP in blood samples obtained from the femoral artery of the nude mice was measured by radioimmunoassay with a PAP kit, using PAP from human prostates for developing radioiodinated antigen and for raising antiseraum (17). The kit was based on the double antibody procedure. Sera from male mice were diluted 20-fold with human sera obtained from female candidates, and sera from female mice were used for assay without dilution. Specimens of 100-µl test samples were incubated with 200 µl of anti-PAP serum (rabbit anti-PAP antiseraum) at room temperature for 20 h. To the mixture, 200 µl of [3H]PAP were added, followed by incubation at room temperature for another 20 h. After incubation, 200 µl of a second antibody (goat anti-rabbit IgG anti-serum) were added to the assay tubes, and the tubes were kept standing for 30 min. The tubes were then centrifuged (1,800 x g, 30 min) at 1°C, and pellets containing the antibody-bound [3H]PAP were counted for radioactivity in a gamma counter (ARC-6000; Aloka, Tokyo, Japan). Statistical analysis of the data was performed by the Student's t-test and Cochran-Cox's method.

Tissue Fractionation. Cytoplasmic and nuclear tissue extracts were prepared at 4°C with a modification of the procedure described by Trachtenberg et al. (18). The tumor was pulverized after freezing in liquid nitrogen and homogenized in 10 volumes of cold TEDG buffer with an all-glass homogenizer 2 times for 30 s with an interval of 30 s of cooling. After filtration through nylon cloth, the homogenate was centrifuged at 800 x g for 15 min. The resulting supernatant was decanted and further homogenized with Polytron PT 30-35 homogenizer (Brinkmman, Westburg, NY). The homogenate was centrifuged at 100,000 x g for 1 h at 4°C with an ultracentrifuge (80P; Hitachi, Hitachi, Japan) to obtain the supernatant cytosol fraction. The crude nuclear pellet obtained from the 800 x g centrifugation was washed twice in 2 volumes of TEDG buffer (800 x g, 15 min) and suspended in 10 volumes of TEDG buffer (105,000 x g, 1 h). Each gradient was collected from the bottom to the top in 3 drop fractions into 40 scintillation vials and counted for radioactivity in 4 ml of Aquasol-2 with a liquid scintillation counter (Ultrobeta; LKB, Sweden). 14C-methylated BSA was run on a separate gradient to determine the approximate sedimentation coefficient. Protein was estimated by the method of Lowry et al. (19).

Saturation Analysis. For saturation analysis of the androgen receptor with DCC, 500 µl of cytosol or nuclear extract were incubated in duplicate with 5 µl of [3H]R-1881 ranging in various concentrations (0.116 nM to 10.6 nM in cytosol and 0.22 nM to 25.2 nM in nuclear extract, respectively) in 2-fold increments, with 10 µl of 1 M sodium molybdate, and with 5 µl of 5 µM of TA for 20 h at 1°C. Then, the mixtures were layered on DCC pellets which were prepared from a mixture of 0.5% activated charcoal and 0.005% dextran in 1.5 ml of TEDG buffer with centrifugation for 10 min at 1,800 x g. The mixtures were stirred and incubated for 30 min at 4°C, followed by centrifugation for 10 min at 2,500 x g. The radioactivity of 100 µl of the supernatant was assayed with the same procedure described in glycerol density gradients, and the data were analyzed according to the method of Scatchard (20).

Competition Assay. Specimens of cytosol (500 µl) were incubated with 5 µl of 2 nM [3H]R1881 in TEDG buffer and 5 µl of TEDG buffer, with or without 20 or 200 nM unlabeled competitors, R1881, testosterone, DHT, estradiol, DES, progesterone, and dexamethasone. The amount of [3H]R1881 bound was determined after 20 h using the same procedure outlined in the saturation analysis. Non-specific binding was subtracted using the amount of 3H-steroid bound in the presence of 100-fold unlabeled analogous competitors.

RESULTS

Serial Transplantation. The growth of HONDA serially transplanted in male mice grew constantly and reached over 1 g in 8 weeks or so with few exceptions which showed some delay in growth. The serial transfers were performed at intervals of 2 to 6 months without failure.

Growth Rate. As shown in Chart 1, tumors transplanted in untreated male mice showed progressive growth, while the tumors transplanted in female mice disappeared about 5 weeks after transplantation. Orchietomy in males performed at 4 weeks after transplantation markedly reduced the growth of the tumor. The growth curve of the tumors transplanted in female mice, followed by treatment with testosterone, showed continuous growth, as observed in untreated male mice. Treatment of male mice with estrogen showed some inhibitory effect of tumor growth; however, the tumor continued to grow. A large deviation in tumor weights in estrogenized male mice was observed.

Histology of Tumors. Histological features of the tumors transplanted in untreated male mice (18th passage) were those of poorly differentiated adenocarcinoma. The histology of the original tumor was well retained in the serially transplanted tumor. In untreated male mice, tumor cells with round hyperchromatic nuclei and pale cytoplasm grew in sheets and cords, showing more or less well-developed glandular lumina (Fig. 1). After orchietomy, the size of the cells decreased, and they were surrounded by stroma of the host, forming small islets of tumor with a PAP kit, using PAP from human prostates for developing radioiodinated antigen and for raising antiseraum (17). The kit was based on the double antibody procedure. Sera from male mice were diluted 20-fold with human sera obtained from female candidates, and sera from female mice were used for assay without dilution. Specimens of 100-µl test samples were incubated with 200 µl of anti-PAP serum (rabbit anti-PAP antiseraum) at room temperature for 20 h. To the mixture, 200 µl of [3H]PAP were added, followed by incubation at room temperature for another 20 h. After incubation, 200 µl of a second antibody (goat anti-rabbit IgG anti-serum) were added to the assay tubes, and the tubes were kept standing for 30 min. The tubes were then centrifuged (1,800 x g, 30 min) at 1°C, and pellets containing the antibody-bound [3H]PAP were counted for radioactivity in a gamma counter (ARC-6000; Aloka, Tokyo, Japan). Statistical analysis of the data was performed by the Student’s t-test and Cochran-Cox’s method.

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cells. Small vacuoles were seen in the cytoplasm, and the frequency of mitosis in the tumor was reduced significantly (Fig. 2). The tumor transplanted in female mice showed severe degenerative changes and was surrounded by histiocytes and foreign body giant cells about 2 weeks after inoculation of the tumor. No tumor cells were observed in these granulomatous tissues (Fig. 3). Histological features of the tumor transplanted in testosterone-treated female mice were essentially identical with those of untreated male mice, showing trabeculae and islets of epithelial cells. However, a glandular arrangement of the tumor cells was seen more frequently than in the control tumor, showing moderately differentiated adenocarcinoma (Fig. 4). Histology of the tumor from estrogenized male mice showed mixed features of the tumors observed in untreated and castrated male mice.

**Prostatic Acid Phosphatase.** Table 1 shows the effects of gender of the hosts and hormonal manipulation on the levels of PAP in the serum of tumor-bearing mice (18th passage). The serum level of PAP in castrated male mice was very low, while characteristic elevation of PAP in female mice treated with testosterone was notable. The levels of PAP in the serum did not depend on the tumor weight, since PAP levels in the estrogen-treated male mice and androgenized female mice showed the marked decrease and increase in PAP, respectively, when the values of PAP were expressed per unit weight of the tumor.

**Androgen, Estrogen, and Progesterone Receptors.** Chart 2 displays patterns of glycerol density gradient centrifugation for androgen, estrogen, and progesterone receptors in cytosol of the untreated male mice (19th passage). The characteristic 8-9S peak, which is estimated from sedimentation constants of BSA and bound to [3H]R1881, is clearly shown. No peak bound at the 4S region is observed. Preincubation with a 100-fold excess of unlabeled R1881 eliminated the 8-9S peak. Glycerol density gradient analysis disclosed no estrogen and progesterone receptors in cytosol of the tumor. The binding of R1881 was specific for the androgen receptors, as indicated by the competition studies (Table 2). DHT and testosterone were good competitive inhibitors of [3H]R1881 binding, and none of the compounds, 17β-estradiol, progesterone, or dexamethasone, succeeded in competing with bound R1881.

**Table 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of mice</th>
<th>PAP (ng/ml)</th>
<th>Wt. of tumor (mg)</th>
<th>PAP/g of tumor (ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>6</td>
<td>131 ± 42a</td>
<td>2375 ± 290</td>
<td>57 ± 15</td>
</tr>
<tr>
<td>Male + estradiol</td>
<td>4</td>
<td>25 ± 6</td>
<td>1375 ± 760</td>
<td>23 ± 13a</td>
</tr>
<tr>
<td>Male + orchietomy</td>
<td>4</td>
<td>0.5 ± 1</td>
<td>145 ± 44</td>
<td>2.3 ± 5.0</td>
</tr>
<tr>
<td>Female</td>
<td>5</td>
<td>1.0 ± 0.1</td>
<td>Trace</td>
<td>ND</td>
</tr>
<tr>
<td>Female + testosterone</td>
<td>6</td>
<td>959 ± 176</td>
<td>2034 ± 345</td>
<td>470 ± 80a</td>
</tr>
</tbody>
</table>

* Values are those of mean serum PAP level divided by mean tumor weight.
* a Mean ± SD.
* Estradiol dipropionate (100 µg) was injected once a week for 4 weeks, starting immediately after transplantation.
* b P < 0.001, F-test.
* c Bilateral orchietomy 4 weeks after transplantation.
* d ND, not determined.
* Testosterone propionate (1 mg) was injected i.m. twice a week for 8 weeks, starting immediately after transplantation.

**DISCUSSION**

Successful heterotransplantation of human prostatic cancer in nude mice has been reported by several investigators (12–14). Shimosato et al. (14) first reported serially transplantable human...
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PAP has been recognized as a marker of prostatic carcinoma for over 40 years (21). The demonstration of PAP in prostatic cancer cells and release of PAP from the tumor cells have been suggested as evidence of hormone responsiveness of the prostate tumors (22). Developmental evidence also indicated that the concentration of PAP in the circulation is under the control of androgen (23). High levels of PAP in tumor-bearing male and testosterone-treated female mice indicate that the release of PAP from the tumor cells is definitely androgen dependent. The cause of the reduction in PAP level after administration of estrogen to male mice is unknown as of yet. However, decrease and increase of PAP levels in HONDA tumor with hormonal manipulations did not depend on the changes of tumor weight. The possible presence of different control mechanisms between hormonal regulation of the tumor growth and production of PAP may not be neglected.

There are discrepancies between the results of levels of PAP in serially transplantable prostatic cancer in nude mice, PC-82, which has been characterized as the PAP-producing tumor. Hoehn et al. (12, 13) have reported 2 lines of androgen-dependent tumors, but biochemical analysis of the hormone dependency, including the evidence for androgen, estrogen, and progesterone receptors, has not been presented so far.

HONDA, established in our laboratory, is an adequate experimental model of human prostatic cancer. Transplantability was 100% in male mice, and the tumor growth was rapid enough to perform experimental studies in vivo. HONDA showed preferable growth in male mice, a decrease in tumor weight as a result of orchietomy, and progressive growth in female mice after treatment with testosterone. These results clearly indicate that HONDA is a hormone-dependent tumor. No growth of the tumor in female mice and inhibition of growth of the tumor in estrogenized male mice indicate some antagonistic effect of estrogen to androgen in regulating growth of the tumor. However, the failure of a relatively large amount of estrogen to arrest tumor growth in male mice and a large deviation in tumor weight of estrogenized male mice suggest that the heterogeneity of the tumor consisted of estrogen-sensitive and insensitive cancer cells.

This assumption may also be supported from the histology of the estrogenized male mice, showing mixed histological features of untreated and orchietomized male mice. Replacement of the tumor cells by granulomatous tissue in female mice indicated that androgen is essential for the initial growth of the tumor by the host. Histological changes of the tumor in the orchietomized mice were reduction in size of the tumor cells, appearance of intracytoplasmic vacuoles, a tendency to form small islets of tumor cells, and proliferation of the mesenchymal element of the host in the stroma of tumor nodules as mentioned. The lack of degenerative changes of the tumor cells, as well as the low number of mitotic figures after orchietomy, suggests that these changes simply indicate a dormant state of the tumor.

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REFERENCES

Fig. 1. Histology of the tumor in male mice, showing cells growing in sheets and cords. Thin arrows indicate mitosis and thick arrow shows glandular lumen. H & E, x 340.

Fig. 2. Histology of the tumor in castrated male mice, showing small islets of cells with vacuolated cytoplasm. H & E, x 340.

Fig. 3. Histology of the small nodule in female mice, in which the tumor became granulomatous tissue with histiocytes and foreign body giant cells. H & E, x 340.

Fig. 4. Histology of the tumor in female mice treated with testosterone, showing trabeculae and sheets of epithelial cells. Glandular arrangement of the tumor cells is prominent. Thin arrow indicates mitosis and thick arrows show glandular lumina. H & E, x 340.
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