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 size.

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). 125I-methylated BSA (60 μCi/mg) was obtained from the Radiochemical Centre (Buckinghamshire, England). Testosterone, DHT, 17β-estradiol, progesterone, dexamethasone, DES, dihydrotestosterone, and activated charcoal were all obtained from Sigma Chemical Co. (St. Louis, MO). Dextran (M, 60,000) was from Nakarai Chemical Ltd. (Kyoto, Japan). Testosterone propionate and estradiol dipropionate were from Teikoku Hormone Co. (Tokyo, Japan), and DES 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) with a trocar subcutaneously in 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.

The abbreviations used are: PAP, human prostatic acid phosphatase; R1881, methyltransferase; R5020, promegestone; BSA, bovine serum albumin; DHT, dihydrotestosterone; DES, diethylstilbestrol; TA, triamcinolone acetonide; TEDG buffer, 10 mm Tris:1 mm EDTA:0.1 mm DTT:10% glycerol (pH 7.4).

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Estradiol dipropionate (100 μg in 50 μl of sesame oil) was injected i.m. into male mice once/week for 8 weeks. Those 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 radio-labeled antigen and for raising antiserum (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 antiserum) at room temperature for 20 h. To the mixture, 200 μl of [125I]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 × g, 30 min) at 1°C, and pellets containing the antibody-bound [125I]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 × g for 15 min. The resulting supernatant was decanted and further homogenized with Polytron PT-10-35 homogenizer (Brinkman, Westburg, NY). The homogenate was centrifuged at 100,000 × g for 1 h at 4°C with an ultracentrifuge (800; Hitachi, Hitachi, Japan) to obtain the supernatant cytosol fraction. The crude nuclear pellet obtained from the 800 × g centrifugation was washed twice in 2 volumes of TEDG buffer (800 × g, 15 min) and suspended in 10 volumes of TEDK buffer (Tissue Fractionation). Cytosol 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 × g for 15 min. The resulting supernatant was decanted and further homogenized with Polytron PT-10-35 homogenizer (Brinkman, Westburg, NY). The homogenate was centrifuged at 100,000 × g for 1 h at 4°C with an ultracentrifuge (800; Hitachi, Hitachi, Japan) to obtain the supernatant cytosol fraction. The crude nuclear pellet obtained from the 800 × g centrifugation was washed twice in 2 volumes of TEDG buffer (800 × g, 15 min) and suspended in 10 volumes of TEDK buffer (1 M Tris: 1 mM EDTA: 0.1 mM diethiothreitol: 0.4 M KCl (pH 7.4). This suspension was homogenized with a Polytron (5 s, setting 5) and centrifuged at 105,000 × g for 1 h at 4°C to yield the supernatant nuclear extract.

Glycerol Density Gradients. One ml of cytosol was incubated with 10 nM [3H]R1881, [3H]R5020, and [3H]estradiol in TEDG buffer, containing 20 mM sodium molybdate with or without a 100-fold excess of unlabeled competing R1881, DES, and R5020, respectively. To measure only the androgen receptor, all assay tubes contained 5 μM TA. After overnight incubation at 0°C, 300 μl of the cytosol were layered on the linear 10 to 30% (v/v) glycerol density gradients and centrifuged at 105,000 × g for 16 h at 4°C. Each gradient was collected from the bottom of the tubes 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]R1881 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 at 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 × g. The mixture was stirred and incubated for 30 min at 4°C, followed by centrifugation for 10 min at 2,500 × 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.
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, treatment indicated below, were measured 8 weeks after transplantation.

Table 1

<table>
<thead>
<tr>
<th>Gender and treatment</th>
<th>Tumor weight (mg)</th>
<th>PAP (ng/ml)</th>
<th>PAP/g of tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>51.6 ± 7.8</td>
<td>13 ± 2.5</td>
<td>4.2 ± 1.0</td>
</tr>
<tr>
<td>Male + estradiol</td>
<td>51.6 ± 7.8</td>
<td>13 ± 2.5</td>
<td>4.2 ± 1.0</td>
</tr>
<tr>
<td>Male + testosterone</td>
<td>51.6 ± 7.8</td>
<td>13 ± 2.5</td>
<td>4.2 ± 1.0</td>
</tr>
</tbody>
</table>

Results from triplicate experiments are expressed as mean ± SD.

Table 2

<table>
<thead>
<tr>
<th>Competing steroid</th>
<th>Concentration (nM)</th>
<th>% displacement of specific binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1881</td>
<td>20</td>
<td>79.2 ± 5.2</td>
</tr>
<tr>
<td>DHT</td>
<td>20</td>
<td>100.0 ± 1.7</td>
</tr>
<tr>
<td>Testosterone</td>
<td>20</td>
<td>76.3 ± 3.7</td>
</tr>
<tr>
<td>17β-estradiol</td>
<td>20</td>
<td>19.7 ± 10.4</td>
</tr>
<tr>
<td>Progesterone</td>
<td>20</td>
<td>72.6 ± 3.7</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>20</td>
<td>13.2 ± 6.5</td>
</tr>
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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...
HUMAN PROSTATIC CANCER IN NUDE MICE

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 tumors 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) reported that PAP was hardly detectable in the tumor-bearing mice. Van Steenbrugge et al. (24) concluded that the tumor line PC-82 contained high levels of PAP and that the concentration of PAP in PC-82 tumor tissue is very insensitive to castration and treatment with testosterone and estradiol. HONDA may be the only model which showed that the release of PAP from the tumor cells is definitely under the control of androgen and estrogen.

The presence of an androgen receptor gives a biochemical basis for direct action of androgen on target cells. Glycerol density gradient analysis of cytosol from HONDA tumor transplanted in male mice revealed a characteristic 8-9S receptor specific for androgen; the binding affinity of the receptor was shown to be high (Kd = 1.2 nM) by Scatchard analysis of results obtained using the DCC method. The androgen receptor disclosed by the latter method was also present in the nuclear extract of the tumor (Kd = 3.0 nM). Competition experiments showed that the binding of [3H]R1881 to cytosolic components was inhibited primarily by androgens and to a much lesser degree by estradiol, progesterone, and dexamethasone, showing that [3H]R1881 binding reflects androgen binding. Estrogen (25-28) and progesterone receptors (27-29) have been found in malignant tissues of human prostate. Estrogen receptor may be involved in the inhibitory mechanism of growth of the tumor by estrogen. However, no measurable amount of estrogen and progesterone receptor was detected in HONDA tumor. Clonal selection of an estrogen-sensitive subline from HONDA tumor is needed to elucidate the discrepancy.

Nuclear androgen receptor content has been suggested to be more valuable in evaluation of androgen dependency of the prostatic cancer (7). However, the interrelationship between hormone sensitivity and androgen receptor content in prostatic cancer cells remains to be examined (7, 30). Presence of a sufficient amount of androgen receptors both in cytosol and in nuclei of HONDA tumor is a useful tool for the research on the role of the receptor in regulation of androgen-dependent growth of the cancer cells. Although HONDA tumor needs further characterization, the evidence described above indicates that HONDA tumor is a valuable model of a hormone-dependent human prostatic cancer.
ACKNOWLEDGMENTS

We are grateful to Etsuko Takahashi for her helpful advice in the performance of receptor assays.

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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