Cytoplasmic Estrogen Receptor in a Pregnancy-dependent Mouse Mammary Tumor (TPDMT-4) and Related Autonomous Lines

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ABSTRACT

Transplantable TPDMT-4 mammary tumors are characterized by pregnancy dependence, growth during pregnancy, regression after delivery, and practically no growth in virgin animals. In this study, tumor pieces were treated with chemical carcinogens in vitro and serially transplanted in virgin mice with increasingly short intervals between passages. Two autonomous sublines were obtained utilizing 4-nitroquinoline 1-oxide (1 mg/ml) and 20-methylcholanthrene (10 mg/ml) and were designated as PIMT-4491 and PIMT-4673, respectively. PIMT-4673 tumors, very similar morphologically to the parent tumors, were composed of small cuboidal epithelial cells which occasionally formed acinar and glandular structures. PIMT-4491 showed similar morphology with groups of spindle cells at early generations but became more sarcomatous at later generations. Cytoplasmic estrogen receptor (ER) was examined by sucrose density and dextran-coated charcoal methods in newly established and parent tumors. ER was undetectable in PIMT-4673. PIMT-4491 had the same levels of ER as did parent tumors up to generation 21, when ER levels began to decrease gradually until they reached 5.1 fmol/mg cytosol protein at generation 50. In TPDMT-4, ER levels varied from 20 to 75 fmol/mg cytosol protein and were not related to growth behaviors. Effects on TPDMT-4 tumors of estradiol and progesterone alone or in combination were investigated at generations 10 and 33. Under all hormonal conditions, late-generation tumors grew better than early ones. ER levels were not significantly different in the two generations, although they tended to be slightly higher at the later generation. Apparent dissociation constants estimated by Scatchard plots were on the order of $10^{-10}$ M in all ER-positive tumors.

INTRODUCTION

A transplantable, pregnancy-dependent mammary tumor line was successfully established from a mammary tumor virus-induced mammary tumor and designated as TPDMT-4 in DDD mice (12). TPDMT-4 tumors are characterized by growth during pregnancy; rapid postpartum regression, which reaches higher peaks in subsequent pregnancies in breeders; and practically no growth in virgin animals. These growth characteristics have been maintained up to transplant generation 35; an increasing degree of variation in response to an antiestrogen and to tocopherols has served as a useful model for studies on endocrine therapies (15, 17, 18). It is generally agreed that estrogens via ER are involved in the growth of hormone-dependent rat mammary tumors (35) and that ER-positive human breast carcinomas respond more frequently to endocrine ablation and antiestrogenic agents than do ER-negative ones (20).

In this work, we tried to establish autonomous sublines by in vivo implantation of TPDMT-4 tumor pieces treated in vitro with chemical carcinogens. We investigated ER in the original, pregnancy-dependent and the newly established, autonomous tumors with a stress on: (a) relationship between cytoplasmic ER and alteration of a dependent tumor toward autonomy; and (b) modulation of cytoplasmic ER levels by different endocrine environments.

MATERIALS AND METHODS

Chemicals and Reagents. $17eta$-[6,7-3H]Estradiol (specific activity, 56 Ci/mmol) and $17eta$-[2,4,6,7,16,17-3H]estradiol (specific activity, 150 Ci/mmol) were obtained from New England Nuclear (Boston, Mass.). Radiopurity of the compounds was checked by thin-layer chromatography on silica gel and was found to be over 97%. Unlabeled $17eta$-estradiol of analytical grade was obtained from Tokyo Kasei Company (Tokyo, Japan) and used for determination of unspecifically bound $17eta$-[3H]estradiol. $17eta$-Estradiol and PG for treatment of mice were purchased from Sigma Chemical Company (St. Louis, Mo.). Norit A was obtained from American Norit Company (Jacksonville, Fla.), and Dextran T70 (Grade D; M.W. 20,000) was from Pharmacia (Uppsala, Sweden). 4NQO was purchased from Wako Junyaku Company (Osaka, Japan) and recrystallized from acetone. MCA was obtained from Fluka AG (Buchs, Switzerland), and MNNG was from Aldrich Chemical Company (Milwaukee, Wis.). Epithioestanol was a gift from Shionogi Seiyaku Company (Osaka, Japan). All other compounds were commercial preparations of analytical grade.

Animals. All mice used were reared in the Laboratory Animal Research Center. DDD, BALB/c, and DDD x BALB/c F1 (hereafter called F1) females, 6 to 7 weeks old, served as recipients of tumors with or without PG. DDD mice of both sexes, 8 to 12 weeks old, were the donors of F1. Mice were allowed

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to have F₂ pellets (Funabashi Nojo Company, Chiba, Japan) and tap water ad libitum. However, NaCl and glucose were added at 0.4 and 2.5%, respectively, to the water for hypophysectomized mice. Mice were killed either by cervical dislocation, for further tumor transplantation, or by decapitation, to obtain tumors for ER assay.

**Induction of Autonomous Tumors.** A TPDMT-4 tumor at transplant generation 4 was obtained from a DDD mouse near the end of her second pregnancy and cut into approximately 2 x 2 x 2-mm pieces. Twenty pieces were placed in a 50-ml flask containing 10 ml PBS alone or PBS combined with 1 μg 4NQO, 10 μg MNNG, or 10 μg MCA per ml and incubated at 37° for 60 min in a water bath shaken 50 times per min. Then, a tumor fragment was taken out, washed thoroughly 3 times with cold PBS on ice, and placed under the skin in the left flank of a 6-week-old F₁ female where there are no mammary glands. Two major diameters bisecting palpable tumors at right angles to each other were measured with vernier calipers once a week for 1 year. The arithmetic mean of these 2 diameters was designated as tumor diameter and used to express tumor growth (14). In each of the control and the treated groups, the largest outgrowth obtained at the end of the observation period was cut into 2 x 2 x 2-mm pieces and used for subsequent implantation into the right inguinal fat pad of a DDD female. Subsequent passages were continued in the same way over increasingly shorter transplant intervals until the take rate reached 100%. All mice were kept as virgins throughout the experiments. To avoid the interference of host cells, F₁ mice were used at the first implantation following the in vitro carcinogen treatment. Finally, 2 autonomous sublines, one in the MCA-treated group and the other in the 4-NQO-treated group, were obtained and designated as PIMT-4673 and PIMT-4491, respectively. These tumors were fixed in 10% formalin solution, processed routinely, sectioned at 5 μm, and stained with hematoxylin and eosin for histological studies.

**Operations.** Bilateral ovariectomy was carried out through an incision in the back; hypophysectomy was done by the transauricular method of Tanaka (32). Successful operations were verified macroscopically and microscopically as described elsewhere (16).

**Tumors for ER Assay.** A tumor piece measuring approximately 2 x 2 x 2 mm was implanted into the right inguinal fat pad of each recipient. CBMT tumors at transplant generation 65 were grown to between 1.1 and 1.5 g in BALB/c virgin mice and included for comparison. The CBMT tumor is a transplantable line isolated from a mammary tumor of a BALB/c-F₂ mouse, and its growth is independent of hormones, ovariectomy, and antiestrogenic effects (14). PIMT-4491 tumors at transplant generations 12, 21, 31, and 50 and PIMT-4673 tumors at generation 14 were grown for 25 to 35 days in DDD virgin mice until they weighed 3.0 to 5.4 g. A TPDMT-4 tumor piece of the same size was placed with or without 3 PI in the right inguinal fat pad of DDD mice. Mice not carrying PI were mated to produce tumor growth. Tumors were obtained at the indicated times during the second or third pregnancy and after the third or fourth parturition. Some multiparous mice were ovariectomized 20 to 30 days after the fourth delivery and killed 70 days after the operation to obtain tumors. Tumors were grown to between 2.0 and 5.5 g for 70 to 90 days after implantation in PI-bearing mice. Some of these mice were ovariectomized and killed 30 or 90 days later. These experiments were carried out between transplant generations 21 and 25.

TPDMT-4 tumors are known to become increasingly more resistant to antiestrogenic effects and less secretory in later generations (14, 15). For this reason, tumors at generations 10 and 33 were grown in PI-bearing mice to investigate whether or not cytoplasmic ER levels under various hormonal conditions vary between the 2 generations. Mice were ovariectomized when tumors grew to between 11 x 16 and 19 x 24 mm within 60 to 80 days of implantation. A 50-μg pellet containing cholesterol alone or 0.16 mg 17β-estradiol, 39.90 mg PG, or 0.16 mg 17β-estradiol plus 39.90 mg PG and cholesterol, was implanted s.c. in the nape of the neck 7 days after the operation. Pellets remained in place for 7 days. During the treatment period, tumor growth was monitored by measuring the 2 diameters (a < b) and was expressed as the percent of change in the volume, as calculated by the formula 0.4a²b (2).

Mice were killed by decapitation to obtain tumors. The excised tumors were trimmed of surrounding fat and normal tissue, washed twice with 0.9% NaCl solution when necessary, frozen on dry ice after drying on filter paper, and stored for at most 1 month at —80° until assay.

**ER Assays.** ER was measured qualitatively using a SDG method described elsewhere (25) and quantitatively using a modification of the DCC methods described by Katzellenbogen et al. (8) and Capony et al. (4). All manipulations to be followed were carried out at 0 to 4° unless otherwise specified. The weighed tumor was minced with scissors and homogenized in 3 volumes of TEM buffer using an Ultra-Turrax homogenizer with several 15-sec homogenization periods, each followed by a 60-sec cooling period. During homogenization, the sample was cooled in an ice jacket. The cytosol was prepared by centrifuging the homogenate at 160,000 x g for 30 min in a type 65 rotor in a Beckman L5-50 ultracentrifuge.

**SDG Method.** Varied quantities of 17β-[3H]estradiol were mixed with 200-μl aliquots of the cytosol and incubated for 1 hr in the presence or absence of excess unlabeled 17β-estradiol or epithiostanol. The mixture was layered on a 5 to 20% linear sucrose gradient prepared in 3.8 ml TEM buffer and centrifuged for 16 hr at 200,000 x g in an SW 56 rotor. The gradient was fractionated from the bottom into scintillation vials to yield 21 equal fractions for counting. Human γ-globulin (7.1S) was used as a sedimentation standard, and its localization was determined by absorbance at 280 nm.

**DCC Method.** Aliquots (200 μl) of the cytosol were added to each of 2 series of tubes, one containing 100 μl TEM buffer alone and the other containing 100 μl 10⁻⁸ M unlabeled 17β-estradiol solution prepared in the same buffer. Then, 100-μl portions of the buffer containing 0.5 to 10 nm 17β-[3H]estradiol were added to each series of tubes. Duplicate reactions were incubated at 0 to 4° for 16 hr for the tumors included in Chart 5. Because tumors from ovariectomized mice gave the same ER levels when duplicate reactions were incubated for 2 to 16 hr, the incubation time was shortened to 4 hr in the experiment shown in Chart 7. Furthermore, because tumors treated with 17β-estradiol alone or 17β-estradiol plus PG revealed undetectable or very low levels of ER under these conditions, exchange assays were conducted to investigate whether or not they have unoccupied ER. Cytosols were pre-treated with a pellet of DCC at 0 to 4° for 20 min and incubated with 17β-[3H]estradiol in the presence or absence of excess...
cold 17β-estradiol at 25° for 4 hr and then at 0 to 4° for 1 hr. In preliminary studies, exchange assays with DCC-treated cytosols were run at 0°, 10°, 25°, 30°, and 37° for between 30 min and 17 hr. Based on the results obtained, a 4-hr incubation at 25° was adopted in consideration of inactivation of TPDMT at 25° was adopted in consideration of inactivation of TPDMT-4 ER at the elevated temperature. The extent of exchange under these conditions was 60 to 80%. After incubation, 500 μl of a suspension of DCC (0.5% charcoal and 0.05% dextran in TEM buffer) were added to each tube. Following an additional incubation for 20 min with periodic agitation, the tubes were centrifuged at 1600 x g for 5 min. The 500-μl portions of the supernatants were added to 10 ml scintillation solution (125 g naphthalene, 7.5 g PPO, and 0.38 g POPOP in 1000 ml dioxane) and counted in a Beckman LS-130 system at 43 to 45% efficiency.

The amount of 17β-[3H]estradiol that specifically bound to ER was determined by subtracting the amount nonspecifically bound (17β-[3H]estradiol bound in the presence of excess, unlabeled 17β-estradiol) from the total amount bound (17β-[3H]estradiol bound in the absence of excess, unlabeled 17β-estradiol). The amounts of specifically bound radioactivity were plotted according to the method of Scatchard (28) to determine ER concentration and the apparent dissociation constant (Kd).

The protein concentration of the cytosol was estimated by the method of Lowry et al. (11) using bovine serum albumin as the standard.

RESULTS

Induction of Autonomous Tumors by Chemical Carcinogen Treatment from TPDMT-4 Tumor (Chart 1). A tumor piece treated with MCA, 4NQO, MNNG, or PBS alone was implanted s.c. in 10 virgin F1 mice. Tumor growth was checked for 12 months by palpation and by measuring the diameters. Palpable tumor growth occurred in 3 mice in each carcinogen-treated group and in 2 in the control group. The average final tumor weight for tumor-bearing mice was 0.23, 0.60, 0.60, and 0.18 g in the MCA, 4NQO, MNNG, and control groups, respectively. At the end of the observation period, the largest tumor from each group was used for subsequent implantation into the right inguinal fat pad of 6 DDD virgin mice; tumor growth was then observed for 4 months. One animal of the MCA group without a palpable tumor died of unknown causes. Tumor growth occurred in 3 of 5 mice in this group and in 4 of 6 mice in the control group. In contrast, none of the 6 untreated, control tumors grew to significant sizes during this period, as was expected from the results of the first transplant generation (in which no palpable tumors appeared before 4 months). In the third and fourth generations, tumor implantation was carried out in the same manner, and tumor growth was monitored for approximately 2 months. Tumor growth occurred in all 5 mice over both generations in the MCA and 4NQO groups. In the MNNG group, however, tumor growth occurred in 4 and 3 of 5 mice in the third and the fourth generations, respectively, with a smaller final tumor weight of 0.35 g in the latter generation. Tumor passage was stopped at generation 4 in this group. Finally, 2 transplantable, autonomous lines were obtained, one from a MCA-treated piece and the other from a 4NQO-treated one. These were designated as PIMT-4673 and PIMT-4491, respectively. These tumors were serially transplanted in DDD virgins and were examined for their response to ovariectomy and hypophysectomy, morphology, and cytoplasmic ER.

The growth of these autonomous tumors was not influenced by ovariectomy, as illustrated in Charts 2 and 3. Because PIMT-4491 tumors contained significant levels of cytoplasmic ER (Chart 5), the effect of hypophysectomy on their growth was investigated at generation 15. We found that they grew at the same rate in virgin and ovariectomized mice and at a slightly slower rate in hypophysectomized mice, probably because of the debilitating effect of the operation on their health (Chart 3). PIMT-4673 tumors also grew in both hypophysectomized mice used (data not shown).

PIMT-4673 tumors were morphologically similar to parent tumors over at least 30 generations. They were composed of cuboidal epithelial cells which occasionally formed acinar and glandular structures (Fig. 1) and were classified as type B according to the scheme of Dunn (5). PIMT-4491 tumors were classified as type A.
showed nearly the same structure at early generations, although groups of spindle cells actively proliferated in many places (Fig. 2). These spindle cells occupied larger areas in later generations (Fig. 3). Finally, tumors showed a fibrosarcoma-like architecture with central necrosis and a small number of cuboidal epithelial cells forming glandular structures in a few places in the periphery (Fig. 4).

**ER in TPDMT-4 Tumors.** The cytosol from a tumor growing during pregnancy was incubated with increasing amounts of 17β-[3H]estradiol and analyzed by a SDG containing no potassium chloride. At lower concentrations of 17β-[3H]estradiol, radioactivity was preferentially incorporated into the fractions in the 8S region. At higher concentrations, these fractions were saturated, and excess radioactivity was recovered in the fractions near the top (Chart 4a). This indicates that the TPDMT-4 tumor contains specific cytoplasmic 8S ER. This was again confirmed by abolishment of a peak of radioactivity in the 8S region when the incubation was carried out in the presence of excess epitiostanol, an antiestrogen which competitively inhibits the binding of 17β-[3H]estradiol and ER of the rat uterus (25) (Chart 4b) or excess cold 17β-estradiol (data not shown). The specific 8S ER was qualitatively demonstrated in a similar manner in all TPDMT-4 tumors included in Chart 5a. These tumor cytosols were measured for ER content by a DCC method followed by Scatchard’s analysis. As indicated in Chart 5a, the cytoplasmic ER level was not influenced by hormonal conditions (e.g., those associated with pregnancy, ectopic PI, delivery, and ovariotomy) although tumors exhibited different growth behaviors (i.e., growth regression and stasis) under these conditions (12, 14, 15).

The dissociation constant calculated from the Scatchard plot varied from 0.10 to 0.65 nM, supporting the specific binding of 17β-[3H]estradiol to ER. The constant appeared to be lower in static tumors from ovariectomized, multiparous, and PI-bearing mice [0.21 ± 0.03 (S.E.) nM] than in growing tumors from pregnant and PI-bearing mice [0.48 ± 0.03 nM].

**ER in Autonomous Tumors.** Cytoplasmic ER was undetectable by either SDG or DCC methods in CBMT tumors; it was detected at an insignificant level by a 500 method but was unmeasurable by a DCC method in PIMT-4673 tumors at generation 9 (Charts 4c and 5b). In PIMT-4491 tumors, however, 8S ER was clearly demonstrated by a SDG method (Chart 4c). The ER contents measured by a DCC method were as high as those of parent tumors and averaged 35.3 fmol/mg protein at generations 12 through 21, but they decreased with advanced generations to an average of 22.0 and 5.1 fmol at generations 31 and 50, respectively (Chart 4b). Apparent dissociation constants varied from 0.2 to 0.6 nM, which were equivalent to those of parent tumors.

**Effect of 17β-Estradiol and PG on Growth and ER of TPDMT-4 Tumors.** PI-bearing mice with grown tumors were ovariectomized and treated for 1 week with 17β-estradiol, PG, or 17β-estradiol plus PG starting 7 days after the operation.
TPDMT-4 tumors have been shown to alter their response to an antiestrogen (14, 15). For this reason, this experiment was conducted at both early and late generations. Tumor growth during the period of treatment in each of the control and treated groups is illustrated as the percent of change in volume in Chart 6. Tumors grew better under all hormonal conditions at generation 33 than under comparable conditions at generation 10. Cytoplasmic ER levels under any given hormonal condition were not significantly different in the 2 generations, although they tended to be slightly higher at the later generation (Chart 7). ER remained at undetectable or very low levels in tumors treated with 17β-estradiol alone or in combination with PG, perhaps due to nuclear translocation. It is, however, interesting that significant levels of ER higher than 10 fmol/mg cytosol protein were detected more frequently in growing tumors under the influence of 17β-estradiol and PG than in static or slowly growing tumors in the presence of 17β-estradiol alone (Charts 6 and 7).

The apparent dissociation constants of control and PG-treated tumors were in the same range at either generation, although they appeared to be lower at the later generation. The apparent dissociation constants were 0.16 ± 0.03 and 0.35 ± 0.03 nM at generations 33 and 10, respectively.

DISCUSSION

Mouse mammary tumorigenesis has been characterized by multistep transformations from normal to neoplastic cells (3, 21). Hyperplastic alveolar nodules and ductal hyperplasias are preneoplastic stages. These lesions are induced by a variety of etiologic agents, viral, chemical, and physical (22). Mammary tumors develop primarily via hyperplastic alveolar nodules in C3H, BALB/c, and BALB/cIc3H mice. In contrast, GR, BR, RII, DD, and DDD mice have another pathway of mammary tumorigenesis via plaques (6, 26). These mouse strains have at least one ancestor of European origin. Plaques are characterized by growth during pregnancy and regression after parturition. They form the histochitecture of the duct when implanted into cleared fat pads in virgin mice (1), but they progress to autonomous tumors during repeated pregnancies or over successive passages in hormone-stimulated mice (31).
Tumors were obtained at the termination of the observation period indicated in Chart 6 and were measured for cytoplasmic ER by DCC method. Cytosols were incubated at 0 to 4°C (○, □) or at 25°C (●, △). Measurements in the same tumor are connected with a solid line. See text for details. Horizontal bars, mean.

related to each other in origin. In this respect, it is interesting that urethane-induced mouse mammary tumors, probably related to ductal hyperplasias, were ovarian dependent and contain ER (18, 19, 35).

In order to obtain autonomous sublines from a TPDMT-4 tumor, tumor pieces were exposed to chemical carcinogens in vivo for a short time and serially transplanted through virgin mice with increasingly short transfer intervals. As a result, 2 autonomous sublines, PIMT-4673 and PIMT-4491, were obtained after treatment with MCA and 4NQO, respectively (Chart 1). These tumors can grow at the same rate in ovariectomized mice as in virgin mice (Charts 2 and 3). This suggests that TPDMT-4 tumor cells may be liable to lose their hormone dependence in response to carcinogenic stimuli under highly selective pressures, although the mechanism is unclear. It is interesting to note that MCA or mouse mammary tumor virus increases the tumor-producing capabilities of nodular cells with additive effects in combination (23).

In both autonomous sublines, cytoplasmic ER was assayed by the SDG and DCC methods. PIMT-4673 tumors did not have significant levels of ER immediately after being established (Chart 5b). In contrast, ER was clearly demonstrated by both methods in PIMT-4491 tumors, although it decreased with passing (Charts 4c and 5b). Morphologically, cuboidal epithelial cells were replaced gradually by spindle cells with passing (Figs. 2 and 3). Tumors became sarcomatous at generation 15 (Fig. 4). The decreased ER content with passing cannot be explained by this variation in cell populations because the ER level showed no significant decline between generations 12 and 21 (Chart 5b). This observation is noteworthy in light of clinical results which show that about one-third of ER-positive breast cancers do not respond to endocrine ablation and therapies (20).

Two inconsistent observations have been published concerning the significance of cytoplasmic ER in ovarian-independent mouse mammary tumors. Shayamala (30) has shown that nuclear translocation of ER is impaired in GRS/A mice, whereas Vignon et al. (34) have indicated that it is not defective in C3H mice. A preliminary study by a SDG method of the nuclear extracts prepared following in vivo injection of 17β-
estadiol indicates that this process is working in the parent line, TPDMT-4, but may not work in the autonomous subline, PIMT-4491.

The TPDMT-4 tumor cytosol in a SDG incorporated radioactivity preferentially into the fractions around 8S when incubated with increasing amounts of 17β-[3H]estradiol (Chart 4a). The 8S peak was completely abolished by addition of excess cold 17β-estradiol or antiestrogen (Chart 4b), indicating binding specificity. This was also supported by high affinity (Kd < 0.7 nm) in a DCC method followed by a Scatchard plot. ER levels did not decline along with tumor regression following parturition in breeders and following ovariectomy in P1-bearing mice (Chart 5a). In this respect, TPDMT-4 tumors are different from dimethylbenzanthracene-induced, hormone-dependent rat mammary tumors, which show a significant decrease in cytoplasmic ER paralleling tumor regression after ovariectomy (7, 9, 33). Cytoplasmic ER levels of ovarian-dependent or -independent mammary tumors from some strains of mice remain unaltered after transplantation into ovariectomized mice (27). Thus, the ovary may not be involved in regulation of ER synthesis by mouse mammary tumor cells. It has been shown that prolactin increases the ER level in cultured rat mammary tissue fragments (10) and human breast cancer cells (29) and in hormone-dependent mammary tumors in vivo (9, 33). However, ER remained at similar levels in ovariectomized, multiparous, and P1-bearing mice (Chart 4a), although the prolactin level was considered to be much higher in the latter.

TPDMT-4 tumors have become more resistant to an antiestrogen, less secretory (14, 15), and have a greater ability to produce PG receptor under the influence of 17β-estradiol as the number of transfers increases (14, 15, 19). These findings led us to investigate the effects of 17β-estradiol and PG alone or in combination on the growth and ER levels of tumors at early and late generations in ovariectomized, PI-bearing mice. We found ER levels to be similar at both generations under any of the hormonal conditions, in spite of the 20 intervening generations. As for tumor growth, it was remarkable that PG alone was comparable to the combination of 17β-estradiol and PG and superior to 17β-estradiol alone in its potential to cause tumor growth at the later generation (Chart 6). In this regard, it is interesting that PG alone enhances the growth of urethane-induced, ovarian-dependent mouse mammary tumors to a greater degree than does 17β-estradiol alone in ovariectomized hosts (36). The mechanism by which PG causes mammary tumor growth in ovariectomized hosts with unmeasurable or insignificant levels of cytoplasmic PG receptor (19, 36) remains to be identified.

The results obtained previously (12–17, 19) and in this study demonstrate that TPDMT-4 tumors are very stable in response to hormones and provide an excellent model system for the study of endocrine therapies and the mechanism of action of hormones in neoplastic growth.

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REFERENCES


Fig. 1. PIMT-4673 tumor at transplant generation 9. Note irregular arrangement of small cuboidal epithelial cells with formation of acinar and glandular structures in some places. H & E, x 275.

Fig. 2. PIMT-4491 tumor at transplant generation 9. Note small cuboidal epithelial cells arranged in the same manner as in Fig. 1 and groups of actively proliferating spindle cells. H & E, x 450.

Fig. 3. PIMT-4491 tumor at transplant generation 15. Note sarcoma-like histoarchitecture and morphology completely different from that in Fig. 2. x 450.

Fig. 4. Periphery of tumor in Fig. 3. Note glandular structures formed by cuboidal epithelial cells. H & E, x 450.
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