Estrogen Receptor Characterization in a Transplantable Mouse Mammary Tumor

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SUMMARY

The estrogen receptor was characterized in a new transplantable mammary tumor line (MXT) which was induced by urethral treatment in C57BL × DBA/2F₁ mice. The tumor is a ductal papillary carcinoma that is ovarian dependent. Treatment of ovariectomized mice with estradiol benzoate results in rapid growth of the tumor. Cytoplasmic and nuclear-bound estrogen receptors were measured by the charcoal adsorption exchange assay and by the [³H]estradiol exchange assay, respectively. Tumor cytosol contained relatively large quantities of receptors, 8 to 9 fmol/mg wet weight, which were depleted from the cytoplasmic compartment after an injection of 2 μg of estradiol. This depletion was accompanied by concomitant and stoichiometric accumulation of receptors by the nuclear fraction. Thus an apparent cytoplasmic-to-nuclear translocation was demonstrated. The affinity of both cytoplasmic and nuclear receptors was similar to that reported by others (~ 1 nM). Receptor binding was specific for estrogens, and other steroids showed no competitive inhibition at concentrations up to 10⁻⁷ M. These results demonstrate that this tumor line contains relatively large quantities of estrogen receptors that are similar to those found in normal tissue. This finding in a tumor that is transplantable makes it an ideal model system for the study of growth control by estrogens in neoplastic tissue.

INTRODUCTION

The relationship of estrogen responsiveness to the presence of specific binding protein or receptor for estrogen has been established by numerous laboratories. The mechanism of action involves the specific binding of the hormone to cytoplasmic receptor and subsequent translocation of this hormone-receptor complex to the nucleus of the cell (1, 4, 8, 12). This mechanism has also been described in both normal and neoplastic mammary tissues (11, 20, 21, 26, 29). The binding of estrogen to its receptor is of limited capacity (saturable) and of high affinity (5).

Animal models that can be used for the study of estrogen-induced responses in relation to receptor-estrogen interactions in normal and malignant mammary tissues are of interest, especially in view of the estrogen dependency reported for some 30 to 50% of human mammary carcinomas (10, 11, 19). These human tumors have been judged estrogen dependent by virtue of their regression response to both "antiestrogen" treatment (9) and endocrine ablative therapy and the correlation of this response to the presence of estrogen receptor in the malignant tissue (6, 15).

This report deals with the characterization of the estrogen receptor in a urethral-induced mammary tumor line (MXT) in C57BL × DBA/2F₁ mice (hereafter called BD2F₁) with respect to its high affinity and saturability of binding, its specificity for estrogens, its correlation to growth responses to estrogens, and its translocatability. We feel that a careful characterization with respect to the above parameters is a necessary and often neglected approach to the study of the estrogen receptor in any tissue. These individual characteristics of the receptor may not be the same in all tissues, either normal or malignant, and should not be assumed to be so. The combination of a transplantable mouse mammary tumor with a translocatable receptor and growth dependence on estradiol is unique. Previously reported mouse mammary tumors containing estrogen receptors were described as estrogen independent with a non-translocatable receptor (25) or with little receptor in the cytoplasm or nucleus (28); others were described as estrogen dependent or independent but were unevaluated with respect to receptor translocatability (22, 23, 27).

MATERIALS AND METHODS

Tumor Induction and Transplantation. The tumors were induced in female BD2F₁ mice carrying a pituitary isograft under the kidney capsule between 4 and 16 weeks of age. Urethan (dissolved in distilled water) was injected i.p. at 20 mg/week for 10 weeks between 6 and 15 weeks of age. Tumors appeared between 12 and 15 months of host age and were serially transplanted with the use of a trocar to implant pieces s.c. into syngeneic mice. Tumors through transplantation generation 5 have been used for these investigations. Samples of each tumor were fixed in Tellysničky’s fixative, embedded in paraffin, processed for routine histological sections, sectioned at 5 μm, and stained with hematoxylin and eosin.

In Vivo Ovarian Dependency. Eleven tumors were picked at random from urethral-treated BD2F₁ mice. Each tumor was minced into 1-cu mm pieces, and samples of each tumor were transplanted s.c. into groups of ten 8- to 10-week-old syngeneic female mice. One week later, 5 of the

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mice in each group were bilaterally ovariectomized. The tumors were measured by vernier caliper weekly and expressed in mm of the longest dimension.

In a 2nd set of experiments, 1 of the tumors (MXT-3590) was minced into 1-cm pieces, and samples were transplanted s.c. into forty 8- to 10-week-old syngeneic female mice. Ten days later, 10 of the mice were bilaterally ovariectomized. Six weeks after transplantation, 10 control mice were bilaterally ovariectomized (Group B), and 10 control mice were left intact (Group A). At the same time, 5 of the mice ovariectomized at 10 days posttransplantation received injections of EB (6 /ug/ml) s.c. twice a week (Group D), and 5 remained as untreated ovariectomized (Group C).

The tumors were measured weekly, and the results are illustrated in Chart 1.

**Receptor Assays.** Radioactive 17\beta-[2,3,6,7-3H]estradiol with specific activity of 98.5 Ci/m mole was purchased from New England Nuclear, Boston, Mass. The buffer used in all assays was TESH.

Receptor assays were performed on tumor tissue of mice that had no injections or that received injections of 2.0 /ug estradiol in 0.1 ml 0.9% NaCl solution. Animals bearing tumors were killed by cervical dislocation. Tumor tissue was removed and placed in cold 0.9% NaCl solution for rinsing of blood and trimming of necrotic tissue. The tissue was then minced and homogenized in ground-glass Kontes homogenizers with motor-driven pestles in TESH buffer. The concentration of tissue used in all experiments was 100 mg per ml buffer. All procedures were carried out at 4° unless otherwise stated. The crude nuclear pellet was prepared by centrifuging the homogenate at 800 x g for 8 min. Pellets were washed 3 times with 1.5 ml TESH as described above and then extracted with 1.25 ml 100% ethanol. The ethanol extract was then added to 5 ml Perma blend scintillation fluid (Packard Instrument Co., Downers Grove, Ill.) and counted as described previously. The concentration of [3H]estradiol specifically bound was determined as described for the charcoal-dextran assay.

The sensitivity of the receptor to various temperatures was evaluated in the above assays with either 5.8 nM (nuclear) or 5.4 nM (cytosol) [3H]estradiol concentrations with or without 100-fold excesses of DES as described above. Assays for nuclear receptor were performed on tumors from animals given injections of 2.0 /ug estradiol 60 min prior to assay. Tubes were then incubated at 4°, 22°, 30°, and 37° for the times shown in Chart 4.

Competition for the binding of [3H]estradiol to nuclear and cytoplasmic estrogen receptor was studied at the [3H]estradiol concentrations described for time and temperature analysis either alone or with approximately 10^-7 M concentrations of DES, corticosterone, dexamethasone, estradiol, progesterone, and testosterone.

**RESULTS**

Mammary tumors appeared between 10 and 15 months of age and were described histologically as well-differentiated ductal carcinomas or papillary ductal carcinomas. The tumors were transplantable s.c. in syngeneic female mice and generally attained a size of approximately 10 mm by 5 to 6 weeks after transplantation. In the 1st set of experiments 10 of the 11 tumors grew very slowly, if at all, in ovariectomized mice (range 2 to 5 mm) by 8 weeks after transplantation. By comparison, tumors in control mice reached 18 to 25 mm in a similar time period. The response to EB is shown in Chart 1. The growth of the tumors was inhibited by ovariectomy, regardless of whether the ovaries were removed when the tumors were small (<2 mm) or large (≥13 mm). EB, given twice weekly, supported the growth of these tumors in ovariectomized mice.

The results of saturation and binding affinity analysis for tumor tissue receptor are shown in Charts 2 and 3. The average mg DNA per mg tumor tissue was 3.3 ± 0.23. Chart 2A shows the saturation of tumor cytosol receptor from an animal that had not received an injection. Chart 2B shows a Scatchard plot (24) of these data revealing a Kd of 0.73 nM with 8.48 fmol receptor binding sites per mg tissue. Chart 2, C and D, shows the saturation curve and Scatchard analysis of cytosol receptor after the s.c. injection of 2.0 /ug estradiol 60 min prior to assay time. The Kd of binding is 1.24 nM with 2.52-fmole receptor sites per mg tissue.

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*The abbreviations used are: EB, estradiol benzoate; TESH, 10 mm Tris, 1.5 mM EDTA, and 12 mM monothioglycerol, pH 7.4; DES, diethylstilbestrol.*
Comparison of several animals not given injections with animals receiving the 2.0-μg estradiol injection as described above demonstrates the phenomenon of receptor nuclear translocation. Receptor binding of [3H]estradiol is shown to be depleted from the cytoplasmic compartment and concomitantly to appear in the nuclear compartment. Table 1 summarizes these data.

The effect of temperature on receptor stability is shown in Chart 4. Chart 4A demonstrates that cytoplasmic estrogen receptor can be optimally assayed at 22° or 30° after 30 to 60 min of incubation. Chart 4B shows that nuclear estrogen receptor is optimally incubated at 37° for 15 to 30 min.

Results of the competition studies are shown on Chart 5. Significant competition for [3H]estradiol binding occurred only in the cases of incubation with estradiol and DES. Corticosterone, dexamethasone, progesterone, and testosterone did not compete for binding.

DISCUSSION

These data provide the preliminary characterization of hormonal dependence and the nature of the estrogen receptor in urethan-induced mouse mammary tumors. Several characteristics of these tumors are noteworthy. The
majority of mammary tumors were ovarian dependent. This is in contrast to mammary tumors frequently found in mammary tumor virus-positive strains such as GR and DDD, which are pregnancy dependent (16, 17). The latter tumors arise from mammary lesions termed plaques, which grow during pregnancy but regress with lactation. It is not understood whether the marked ovarian dependency of the hybrid tumors described herein is due to the strain combination, the nature of the carcinogen, or the morphological type of mammary tumor. However, similar ductal carcinomas induced by 7,12-dimethylbenzanthracene in BALB/c mice are not notably ovarian dependent. The histological characterization of these tumors as papillary ductal carcinomas and well-differentiated ductal carcinomas also make them an attractive model for further study, since human mammary carcinoma is likewise of ductal cell origin (18).

The estrogen receptor in this tumor line was readily demonstrated, and it exhibited the generally accepted criteria and characteristics previously described for estrogen receptors in other tissues. The presence of the receptor in this line agrees well with its apparent estrogen sensitivity. This is in contrast to previously described mouse mammary tumors in mammary tumor virus-positive C3H female mice which have been shown to be estrogen independent. This hormone independence was correlated with the inability of the cytoplasmic receptor to undergo nuclear accumulation (25). Other estrogen-independent mouse mammary tumor lines contain little estrogen receptor in either the cytoplasmic or nuclear compartments (22, 23, 27, 28).

The average $K_d$ for estradiol binding to receptor in the urethan-induced tumor was $1.23 \pm 0.23$ nM. This value is in good agreement with other $K_d$ values reported for normal and neoplastic mammary tissues. Richards et al. (23) reported that estradiol receptors of normal lactating mammary gland from a survey of various murine strains had a $K_d$ value ranging from 0.28 to 0.54 nM and that those of mammary tumors ranged in $K_d$ from 0.35 to 0.65 nM. The $K_d$ values for normal lactating and tumor tissues from strain C57BL (1 of the parental strains of the hybrid mouse used in this investigation) were 0.35 and 0.65 nM, respectively. The capacity of cytosol receptor binding reported in this study is higher than that reported by Richards et al. (23). MXT contains 8.48 fmoles/mg of tissue, whereas normal and tumor tissues from the earlier study (23) ranged from 0.63 to 1.80 fmoles/mg of tissue.

In general, the other characteristics of the estrogen receptor in this tumor line appear similar to those previously reported for receptors in other tissues. The temperature dependency of binding and exchange of $[^3H]$estradiol or cytoplasmic and nuclear receptor is in agreement with that reported in other estrogen-dependent tissues (5, 7, 13). Hormone specificity of the MXT estrogen receptor is also in keeping with that described for other estrogen-dependent tissues (5). Displacement of estrogen can be accomplished only with competition by other estrogens.

This study of a new transplantable tumor has demonstrated that it is dependent on estrogen for growth, contains relatively large quantities of translocatable estrogen receptors with characteristics similar to those described for receptors of other estrogen-dependent tissues, and in addition has been identified by histological evaluation as a papillary ductal carcinoma. According to these criteria, MXT provides an excellent model system for the study of estrogen action in a neoplastic tissue.

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