Search for Estrogen Receptors in Human Meningioma Tissue Sections with a Monoclonal Antibody against the Human Estrogen Receptor

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Abstract

Meningiomas are rich in progesterone receptors, whereas estrogen receptors (ER) are virtually undetectable. The present experiments were performed to evaluate whether the absence of ER from the majority of human meningioma cytosols can be attributed to: (a) occurrence of only a small number of ER-positive cells in an otherwise ER-negative tissue; (b) resistance of nuclear receptors to extraction; or (c) impairment of steroid binding. Twenty-one specimens were selected from our total series of 46 meningiomas. Based on cytosol assays, five of these meningiomas were considered to be ER positive (10–42 fmol/mg protein) and five had marginal ER concentrations (4–9 fmol/mg protein), whereas the remaining tissues were ER negative. For comparison, human breast cancer tissues were used. Tissues were sectioned at 6 μm and stained immunocytochemically using a monoclonal antibody against the human ER. The breast cancer specimens showed specific nuclear staining in part of the tumor cells. The sensitivity of the immunocytochemical assay was found to be sufficient to detect staining in breast cancer tissues containing as little as 17 fmol ER/mg cytosol protein. No specific staining was observed in meningioma tissues. It is concluded that the majority of meningiomas are truly devoid of ER and that the estrogen binding agent detected in low concentration in some meningioma cytosols is immunologically different from the human breast cancer ER. The presence of progesterone receptors in meningioma apparently does not require the continuous presence of ER.

Introduction

Epidemiological observations regarding the incidence of meningiomas, the aggravation of their symptoms during pregnancy (1), and an association between meningiomas and breast cancer (2) have led to the hypothesis that human meningioma is a target tissue for female sex steroids. Consequently, attempts have been made to identify sex steroid receptors in meningioma tissue. Initially only ER (3) were studied, but it was soon recognized that the frequency and concentration of progesterone receptors (PR) exceeded those of ER (4–6). With respect to the presence of high concentrations of PR in meningioma there appears to be a consensus in the literature, but there still is disagreement concerning the presence of estrogen receptors. It has recently become clear that part of this disagreement has to be attributed to differences in methodology used by the different investigators. In particular, the use of Scatchard plot analysis appears to be important in this respect, since investigators using single point assays tend to find a higher frequency of ER in meningioma than investigators using Scatchard plot analysis (6, 7).

The synthesis of PR is generally considered to be under estrogenic control as in breast or uterine tissue. A functional ER is thought to be required for PR synthesis. Meningiomas thus appear to behave exceptionally in this respect and, therefore, a more detailed search for the presence of ER in these tissues is warranted. Theoretically, the lack of ER in meningioma cytosol can be explained by a number of factors other than the mere absence of the ER. Among these are metabolism of ligand during the incubation, proteolytic degradation of the ER, irreversible occupancy of ER by endogenous ligand, resistance to extraction of ER which may be located predominantly in the nuclei of their target cells (8, 9), and the presence of a limited number of ER-positive cells in an otherwise ER-negative tissue. The present study was performed to evaluate these possibilities with the aid of an immunocytochemical method using monoclonal antibodies to the human estrogen receptor, which has recently become available (10).

MATERIALS AND METHODS

Assay of ER and PR in cytosol. Intracranial meningioma tissues were obtained and ER and PR were assayed in cytosols with the dextran-coated charcoal method and Scatchard plot analysis as described before (6, 11). A cytosol was considered to be receptor positive when a statistically significant correlation was observed in the Scatchard plot, the calculated dissociation constant was less than 5 nmol/liter, the protein concentration of the cytosol exceeded 1 mg/ml, and the receptor content was higher than 10 fmol/mg protein. Only for the evaluation of the results of the immunocytochemical ER assay, ER levels of 4–9 fmol/mg protein were classified as "marginal" when the other three criteria were met. Human breast cancer tissues with a wide range of ER concentrations which were submitted for routine receptor assay were used for comparison. Remainders of tissues were stored without being thawed at −70°C until further processing.

Immunohistochemical Assay of ER. Twenty-one meningioma tissues with different ER concentrations were selected for this study. Only some of the ER-negative tissues were included in this selection. Frozen meningioma and breast cancer tissues were sectioned at 6 μm, fixed on microscopic slides which had been treated with a tissue adhesive, and processed for immunocytochemical staining of ER as recommended by the manufacturer of the reagents (ER-ICA; Abbott Laboratories, Chicago, IL). Briefly, specific staining was blocked and sections were incubated with either the anti-ER-antibody or a control monoclonal antibody which lacks affinity for the estrogen receptor. Thereafter, the sections were incubated with a bridging antibody, a peroxidase-antiperoxidase complex and diaminobenzidine substrate for the development of staining. Countertaining was done very briefly (5–10 s) with 1:10 diluted Harris’ hematoxylin. After dehydration the sections were embedded.

Centrifugation through Sucrose Gradients. In one experiment, cytosols of a human breast cancer specimen (496 fmol ER/mg protein) and the meningioma with the highest ER level as determined by Scatchard plot analysis (42 fmol/mg protein) were incubated overnight at 4°C with 5 nmol [3H]estradiol/liter either in the presence or absence of a 200-fold excess radioinert DES. Unbound steroid was removed by a 10-min treatment with dextran coated charcoal. Thereafter, 50 μl of the specific and aspecific monoclonal antibodies supplied with the ER-ICA reagent kit were added to 200-μl samples of labeled cytosol and incubated for 1 h. The samples were then centrifuged for 2.75 h at 65,000 rpm in a Beckman VTi-65 rotor through a 10–30% sucrose gradient in cytosol buffer containing 0.4 M KCl (12).

Results

Scatchard plots obtained after ER and PR assay in a meningioma cytosol obtained from a 75-year-old female patient are...
shown in Fig. 1. This tissue was found to be positive for both ER and PR. In most cytosols, however, specific binding of estradiol was not observed as shown in Table 1.

With the immunocytochemical assay, specific nuclear staining was observed in certain parts of the breast tumor specimens. The overall results of the immunocytochemical assays for ER are shown in Table 2. In this table, the results of the individual breast cancer specimens are given. Heterogeneity in the nuclear staining pattern was most striking. One specimen (B-5) which was assayed on two occasions showed different results. Specimen B-7, by contrast, gave a consistent result in three separate assays.

Based on the results of the Scatchard plot analyses, meningioma specimens were divided into three groups, i.e., ER negative (M-1 to M-11); ER positive (M-17 to M-21; ER > 9 fmol/mg protein); and those with marginal ER levels (M-12 to M-16; ER 4-9 fmol/mg protein). ER were not detectable immunocytochemically in any of these groups (Table 2). In one meningioma specimen, intensely stained cells were seen after incubation with the specific ER antibody, as well as with the control monoclonal antibody. These cells are thought to be blood borne; e.g., macrophages.

To further substantiate the absence of immunologically detectable ER in meningioma, radiolabeled cytosol of the meningioma specimen with the highest ER level (42 fmol/mg protein) was incubated with the monoclonal antibodies and centrifuged through a 10-30% sucrose gradient. In a breast cancer specimen which was used for comparison, the binding of the tracer was completely abolished by the addition of the radioinert DES, and the incubation with the monoclonal ER antibody caused a marked shift in the sedimentation constant (4S-8.5S) as shown in Fig. 2. Sixty-two % of DES displaceable radioactivity was shifted by the antibody. For the meningioma specimen, addition of DES only partly displaced the radioactivity and incubation with the ER antibody revealed only a very small peak sedimenting at approximately 7.5S. This small peak did not appear after the incubation with the control antibody (Fig. 2). The amount of radioactivity shifted by the ER antibody accounted for 51% of DES displaceable radioactivity.

Discussion

The data obtained with the breast cancer specimens demonstrate that the sensitivity of the immunocytochemical assay for ER is sufficient to detect stained cells in breast cancer specimens containing as low as 17 fmol ER/mg cytosol protein. Based on the apparent ER levels as found in meningiomas by Scatchard plot analysis, the sensitivity of the ER-ICA should therefore be sufficient for application on meningioma tissue. No specific nuclear staining was found, however, with this type of tissue. Because meningioma tissue is relatively rich in PR, this implies that the antibody used for the detection of ER does not cross-react with human PR.

In this study, the breast cancer specimens were used solely as positive control tissues in assays which were likely to yield large numbers of negative results. Although indicative, the present data (Table 2) do not allow conclusions with respect to the relation between quantitative ER levels and the percentage of stained cells or the intensity of staining. To arrive at definite conclusions, especially with regard to the striking heterogeneity observed in the breast cancer specimens, larger series of samples will have to be judged.

Our present results confirm the absence of ER from human meningioma tissue. We have demonstrated that on the basis of Scatchard plot analyses only 9% of meningiomas have ER levels exceeding 9 fmol/mg protein and thus were considered ER positive, whereas 90% are PR positive. Moreover, PR levels in meningioma are comparable to those in breast cancer, whereas
the ER levels found in meningioma were very low. The steroid specificity (6) and the nuclear localization (11) of the progestin binder in the meningioma tissue indicate that this is a true PR. It thus appears that the presence of PR in meningioma does not require the continuous presence of ER.

On the other hand, a putative ER might escape detection in the cytosol for several reasons. First, the receptor may be proteolytically degraded or the tracer may be metabolized to a compound with less affinity during the incubation. These possibilities are unlikely since the ER concentration of an ER-positive calf uterine cytosol was not affected by the addition of ER-negative meningioma cytosol (data not shown). Secondly, the ER which may reside permanently in the nuclei (8, 9) might resist extraction with hypotonic buffer. To evaluate this possibility, ER were also assayed in 0.6 M KCl extracts of meningioma nuclei (13). Seven extracts showed no binding at all, while one contained only 6 fmol ER/mg nuclear extract protein with a calculated $K_d$ of 0.4 nmol/liter. Moreover, no immunocytochemical evidence to indicate nuclear localization was obtained. Therefore, we concluded that it is very unlikely that nuclear retention of ER has prevented its detection in meningioma cytosols. Other possibilities, such as impaired steroid binding to an otherwise intact ER molecule or presence of only a few ER-positive cells in an essentially ER-negative tissue, appear also to be ruled out on the basis of our results with the immunocytochemical assay. Finally, ER may be present in meningioma cells in concentrations below the detection limit of the ER-ICA. Although this possibility does not seem very likely, it cannot be ruled out at present.

In summary then we conclude that the majority of meningiomas are truly devoid of estrogen receptors and that the estrogen binding agent detected in low concentrations in some meningiomas is immunologically different from the human breast cancer estrogen receptor.

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