Estrogen Receptor and Hormone Responsiveness of Medullary Thyroid Carcinoma Cells in Continuous Culture

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ABSTRACT

We have examined the estrogen responsiveness and estrogen receptor in medullary thyroid carcinoma using a model of an established human cell line, TT. TT cells bind [3H]estradiol with high affinity. Scatchard analysis reveals a single class of binding site with a concentration of 173 fmol/10^6 cells and a dissociation constant of 2.1 x 10^-9 M, values which are comparable to those of a well established model cell line for estrogen responsiveness, MCF-7 human breast cancer cell line. Estradiol in physiological concentrations moderately stimulated TT cell proliferation, whereas in pharmacological concentrations it markedly inhibited cell growth. [3H]Thymidine incorporation into acid-insoluble material was also stimulated following a 5-day treatment with 5 x 10^-8 M estradiol. Tamoxifen at a concentration of 1 nM reduced cell proliferation by 43-48% after 5-7 days of treatment. The growth suppression induced by tamoxifen was reversed by addition of 10 nM estradiol. This is the first report of estrogen growth stimulation and tamoxifen growth inhibition of a tumor cell line derived from human medullary thyroid carcinoma.

INTRODUCTION

Medullary thyroid carcinoma is a cancer of the thyroid C-cells. This type of tumor is commonly capable of producing multiple polypeptide hormones, but calcitonin is the most specific and sensitive marker of MTC (1). It has been demonstrated (2-4) that normal thyroid C-cells are responsive to exogenous estrogens, although whether the estrogen action is mediated through the conventional estrogen receptor has not been clarified. However, it has never been reported before that estrogens play a role in the proliferation of MTC. In this study, we investigated ER and estrogen responsiveness of MTC on the basis of two hypotheses: (a) normal thyroid C-cells possess ER, and the transformed cells retain the ability to synthesize the receptor proteins; and (b) thyroid C-cells acquire ER during the process of malignant cell transformation. The first hypothesis is generally accepted as an explanation for the presence of ER in tumors that derive from established target tissues for estrogens, e.g., breast and endometrial cancers, whereas the latter hypothesis was proposed as an explanation for the existence of ER in tumors that are not traditionally considered hormonally sensitive. These include carcinomas of the lung (5, 6), pancreas (7, 8), colon (9-14), bone (15), and kidney (16), malignant melanoma (17-20), and lymphomas (5). Because a cell culture system provides a direct means for investigating hormonal responses, the availability of a continuous cell line established from human MTC has prompted us to study its responses to estrogen and antiestrogen.

MATERIALS AND METHODS

Ligands and Chemicals. Radioactive 17β-[2,4,6,7-3H]estradiol (107 Ci/mmol) was obtained from Amersham/Searle Corp. (Arlington Heights, IL) and [3H]thymidine (6.7 Ci/mmol) was purchased from Du Pont NEN (Boston, MA). Tamoxifen citrate, 17β-estradiol, progesterone, testosterone, and dexamethasone were obtained from Sigma Chemical Co. (St. Louis, MO). Scintiverse II scintillation fluid was purchased from Fisher Scientific (Houston, TX).

Human Medullary Thyroid Carcinoma Cell Line. The established human medullary thyroid carcinoma cell line TT (21) was a gift from Dr. Susan S. Leong, Roswell Park Memorial Institute, Buffalo, NY. This cell line was maintained as a monolayer culture in RPMI 1640 medium containing 10% fetal calf serum and 2 mM glutamine and in a humid atmosphere in the presence of 5% CO2.

Whole-Cell ER Assay. ER was measured by a whole-cell method established by Shafie and Brooks (22) with modifications (23). Cells were plated in a density of 5 x 10^4 cells/glass scintillation counting vial in 5 ml of RPMI 1640 medium containing 10% charcoal-stripped FCS and 2 mM glutamine. ER assay was performed on these cells after a 4-day incubation at 37°C; at that time, cells were in their exponential phase of growth. After washing with 1 ml of RPMI 1640 medium, the cells in each vial were added to 0.5 ml serum-free RPMI medium containing 0.5-5 nM [3H]estradiol. Nonspecific binding of [3H]estradiol was determined by simultaneous inclusion of a 200-fold excess of unlabelled estradiol. After incubation at 37°C for the periods specified (normally 30 min), the reaction was terminated by adding 2 ml ice-cold medium, and then the medium was aspirated. The cells were washed twice with chilled 0.9% NaCl solution and then extracted with 2 ml ethanol at room temperature. An aliquot of the ethanol extract was counted in Scintiverse II for radioactivity.

Effects of Estradiol and Tamoxifen on Cell Proliferation of TT. Cells were plated into 60-mm Petri dishes in 5 ml RPMI 1640 medium containing 10% charcoal-stripped FCS in a cell density of 5 x 10^4 cells/dish. Twenty-four h later, the culture medium was replaced with fresh phenol red-free RPMI medium containing either 10% stripped FCS (control) or stripped FCS plus various concentrations of estradiol. In experiments in which the effect of tamoxifen was determined, the cells were grown in RPMI medium containing phenol red and 5% FCS without (control) or with tamoxifen. In both cases, the media were changed every 48 h. At the times indicated, duplicates of Petri dishes of each group were processed for determination of cell number. Cells were harvested from Petri dishes with 0.05% trypsin-0.02% EDTA, and the monodispersed cells were counted with a Coulter Counter (Model ZBI; Coulter Electronics, Inc., Hialeah, FL).

[3H]Thymidine Incorporation into Acid-Insoluble Material. Cells were plated into 60-mm Petri dishes and grown in regular medium for 24 h and then in medium containing stripped FCS with or without hormone treatment. At the times indicated, 2.5 μCi of [3H]thymidine were added to each Petri dish, and these were incubated at 37°C for 3 h. At the end of incubation, the dishes were chilled on ice, and the medium was withdrawn. The cells were added to 2 ml of cold 5% TCA solution. After incubation at 4°C for 30 min, TCA was removed, and 2 ml of 0.5 N NaOH were added. After incubation at 37°C for 15 min, the solution was added to 6 ml of 20% TCA, and the pellets were obtained by centrifugation at 10,000 x g. The pellets were washed twice with 5 ml cold 5% TCA and then boiled in 2.5 ml of 5% TCA for 15 min. The supernatants were counted in Aquasol for radioactivity. Duplicates of cell samples were chilled immediately after the addition of [3H]thymidine and precipitated with TCA to serve as control for nonspecific counts.

RESULTS

Fig. 1 shows the kinetics of specific estradiol binding to TT cells. [3H]Estradiol bound to TT relatively rapidly; half-max-
ESTROGEN-RESPONSIVE MEDULLARY THYROID CARCINOMA CELLS

Fig. 1. Intracellular specific binding of [3H]estradiol as a function of time in the TT cells. Cells in the exponential phase of growth were incubated with 8 nM [3H]estradiol at 37°C for the time periods indicated. Specific binding is the difference between total binding in the absence of unlabeled estradiol and the nonspecific binding in the presence of a 200-fold excess of estradiol. Nonspecific binding ranges from 29 to 41% of total binding in this series of experiments. Points, means of 6 determinations in 3 separate experiments. Bars, SE.

Fig. 2. Specific binding of [3H]estradiol and Scatchard plot of the dissociation constant (Kd) and binding capacity in the TT cells. Cells (0.5 x 10^6) in the exponential phase of growth were incubated with [3H]estradiol, in concentrations indicated, at 37°C for 30 min in the absence or presence of a 200-fold excess of estradiol to determine specific binding. Points, means of 6 determinations; bars, SE.

Because tamoxifen antagonizes estrogen effect in estrogen-responsive cells, including normal uterus and breast carcinoma, and because tamoxifen exerts its effect at least partially through an ER-mediated mechanism (26-31), we further examined whether the TT growth is affected by treatment with tamoxifen. As shown in Fig. 4, 1 µM tamoxifen inhibits TT growth by 48% after 7 days exposure. To ascertain whether the suppression of cell proliferation caused by tamoxifen can be reversed by a subsequent treatment of the cells with estradiol, cells were first incubated with 1 µM tamoxifen for 7 days, and the treatment was followed by a second incubation during which 10 nM estradiol was also added to the growth medium. Fig. 4 shows that the tamoxifen-inhibited cell growth was reversed by the subsequent incubation in which estradiol was present, indicating that the ER in TT cells is involved in regulation of cell growth.
DISCUSSION

Although the role of estrogens and their receptors in regulation of neoplastic growth in breast cancer is well documented, this is the first report showing that estrogen and an antiestrogen are involved in the growth regulation of medullary thyroid carcinoma cells. Our results indicate that the growth of TT cells can be moderately stimulated by physiological concentrations of estradiol and greatly inhibited by tamoxifen. TT cells bind estradiol specifically and with high affinity. The dissociation constant, which is estimated to be $2.1 \times 10^{-9}$ M, is within the range of serum estradiol concentrations of premenopausal women. It is, therefore, tempting to suggest that the estrogen-binding proteins in TT cells represent functional receptors.

The response to estradiol of TT cells, although real ($P < 0.02, 5 \times 10^{-9}$ M), was small by comparison to that in human breast cancer cells (30, 32–34). The poor stimulation of cell proliferation induced by estradiol in TT cells cannot be explained by the masking estrogenic effect of phenol red in the culture medium (34, 35), because in this study cells were grown in phenol red-free medium. The lower magnitude of stimulation led to consideration of whether the observed estradiol effect was mediated via the conventional receptor mechanism; for example, mechanisms other than binding of the receptor-ligand complex to DNA could be operative in these cells and be responsible for the stimulation of cell proliferation. In this regard, the observation that tamoxifen-induced inhibition of cell proliferation can be reversed by estradiol would favor the suggestion that the estradiol effect is mediated via the conventional receptor mechanism. Alternatively, culturing conditions are important for optimal response to estradiol (36, 37), and it is possible that optimal conditions were not achieved in our experiment. This may therefore explain the lower magnitude of stimulation observed in this study. The inhibitory effect of higher doses of estradiol on cell proliferation, on the other hand, is most likely nonspecific to the ER status and is consistent with the observations for other ER-positive cell lines, including MCF-7 (30).

The results of the tamoxifen growth-inhibitory effect on TT cells are similar to those reported for MCF-7 breast cancer cells. Growth of MCF-7 cells in vitro is inhibited by exposure to $10^{-10}$ M tamoxifen and maximally by $10^{-6}$ M tamoxifen (38). The inhibitory effect produced by tamoxifen in concentrations of $10^{-7}$ to $10^{-10}$ M is reversed following exposure to $10^{-9}$ to $10^{-8}$ M estradiol (38–40). In our study, 1 nM tamoxifen suppressed TT growth by 48%, and this growth inhibition was reversible if 10 nM estradiol was also included in the medium during the incubation. Thus, our results are consistent with those of others (38–40) and indicate that the ER system in TT cells is functioning.

The presence of estrogen receptor in MTC is of potential interest from a therapeutic point of view because it has been shown that tamoxifen has a very effective antitumor effect on estrogen-responsive tumors and has low systemic toxicity. At present, MTC is usually treated by surgery. However, these tumors tend to infiltrate surrounding tissues (41) and therefore may not be completely removed. Also, metastatic MTC is resistant to irradiation and to anticancer agents. It is possible that the presence of functional ER may predict the response of hormonal therapy. Furthermore, it has been shown that the cytotoxic efficacy of chemotherapeutic drugs can be enhanced by concomitant treatment with estrogens and antiestrogens in breast cancers (40, 42–47). Further studies would be required to ascertain whether a combination of hormonal therapy and chemotherapy could be of potential benefit to the treatment of MTC.

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