Inhibitory Effects of Estrogen on the Growth of a Human Esophageal Carcinoma Cell Line

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ABSTRACT

In order to accurately determine sex hormone dependency and hormone responsiveness in human esophageal carcinoma, the effects of sex hormones on the growth of esophageal carcinoma cell lines, KSE-1 and KSE-2 cells, were examined in vitro and in vivo. Cell proliferation of cultured KSE-1 cells was inhibited by treatment of estradiol and enhanced by dihydrotestosterone (DHT), whereas KSE-2 cells were unaffected by these sex hormones. The heterotransplanted tumors of KSE-1 cells in nude mice possessed estrogen receptor (ER) and androgen receptor (AR), while the tumors of the KSE-2 cells had neither ER nor AR. When the tumor growth rates and serum hormone levels were monitored during the continuous administration of either estradiol or DHT, no significant differences were observed in either the serum hormone levels or tumor growth rates between male and female mice. The administration of estradiol significantly inhibited the growth of ER-positive and AR-positive KSE-1 tumors in both males and females in conjunction with an increase in the estradiol levels and a decrease in the DHT levels in the serum. However, the growth of ER-negative and AR-negative KSE-2 tumors was not influenced by either estradiol or DHT administration. These results suggest that the in vitro growth of human esophageal carcinoma cells with sex hormone receptor is influenced by circulating hormone levels and can be manipulated by systemic estradiol administration.

INTRODUCTION

Clinical data on esophageal carcinoma reveal the different profiles between men and women; the occurrence is more frequent in men (1, 2) and the prognosis is more favorable in women (3, 4). We have thus far demonstrated that the survival rate after surgery in women exceeds those of men (5) and that esophageal carcinoma in women shows a more favorable prognostic factor of DNA aneuploidy patterns reflecting the malignant potential of the tumor (6).

Furthermore, we have recently found that an established esophageal carcinoma cell line, KSE-1 cells derived from a male, have both ER and AR and that their cell proliferation in vitro was affected by the administration of sex hormones, being inhibited by estradiol and enhanced by DHT (7). These findings suggest that the biological characteristics and growth patterns of esophageal carcinoma may be related to sex hormones and increase the possibility that hormone therapy could be applicable to the treatment of esophageal carcinoma.

In order to substantiate and broaden these assumptions, in the present study the effects of systemic administration of sex hormones on the in vitro growth of esophageal carcinoma were determined in two types of an ER-positive, AR-positive cell line and an ER-negative, AR-negative cell line.

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MATERIALS AND METHODS

Chemicals. EMEM without phenol red (8) was purchased from Nissui Pharmaceutical Co., Ltd., and FCS was obtained from M. A. Bioproducts. Estradiol (17β-estradiol) and DHT were purchased from Sigma Chemical Co. and dissolved in ethanol and then directly diluted with Hanks' solution before use.

Primary Culture and the Establishment of Cell Lines. Human esophageal cancer tissues were obtained from the surgically resected specimens of metastasized lymph nodes, those of a 68-year-old Japanese man for KSE-1 cells and those of a 73-year-old Japanese woman for KSE-2 cells, respectively. The methods used for the primary cultures of these two cell lines have been described previously (7), in brief; the viable tissues were minced and a single cell suspension was prepared by mechanical dissociation and enzymatic digestion with 0.14% collagenase type 1. The primary cells were cultivated in EMEM supplemented with 20% heat-inactivated FCS, 100 μg/ml of sodium piperacillin (Toyama Chemical Co.), 5 μg/ml of gentamicin (Schering Co.), and 5 μg/ml of amphotericin B (E. R. Squibb & Sons). The grown cells were harvested by trypsinization and serially subcultured with a 1:4 split ratio. Cloning was performed at around the 10th passage and the resulting cell lines were then used for the experiments at the 45th-50th passage.

Effects of Estradiol and Testosterone on in Vitro Cell Growth. The cell growth responses to estradiol or DHT were examined by measuring the number of cells in the KSE-1 and KSE-2 cell cultures. The cells at the logarithmic growth phase were seeded into a 50-mm tissue culture dish at a density of 5x10^4 cells/dish and incubated at 37°C in a 5% CO₂/95% air atmosphere for 48 h. The cells were then washed five times with serum-free medium and fresh EMEM, containing 10% DCC-treated FCS, was added, either with or without various concentrations of estradiol or DHT. After hormone treatment for 48 h, the cells were removed from the plates using 0.25% trypsin solution and then the cells were counted by Coulter Counter. The percentage of cell growth after treatment of estradiol or DHT relative to cell growth in the control culture was then determined.

Heterotransplantation. Male and female BALB/c- nu/nu mice, age 4 weeks and weighing 22–24 g (Kurea Co., Ltd., Tokyo, Japan), were used. The cell suspension containing 1x10^6 cells in 0.5 ml of EMEM was transplanted s.c. into the dorsal aspect of each mouse. Two weeks after inoculation, when the size of the tumor reached about 5 mm in diameter, hormone administration was started.

Experimental Groups and Drug Administration. The nude mice bearing KSE-1 or KSE-2 tumors were divided into 6 groups according to sex and treatment as follows: nontreated males; nontreated females; estradiol-treated males; estradiol-treated females; DHT-treated males; and DHT-treated females. Estradiol (10^-5 mg/body) or DHT (10^-4 mg/ body) was then injected i.p. every other day for 24 days. Equal volumes of saline were administered to the nontreated groups as a control at the same time intervals. The size of the tumors was measured every 3 days and the tumor volume (V) was expressed as

\[ V = \frac{a \times b^2}{2} \]

in which a is the largest diameter, and b is the smallest diameter.

2 The abbreviations used are: FCS, fetal calf serum; EMEM, Eagle's minimum essential medium; DCC, dextran-coated charcoal; DHT, 5α-dihydrotestosterone; ER, estrogen receptor; AR, androgen receptor; TESH-glycerol buffer, 0.02 M Tris-HCl-0.0015 M EDTA-0.012 M monothioglycerol-5% glycerol, pH 7.8.
Measurements of Serum Hormone Levels. In order to examine the chronological change in serum hormone levels after estradiol or DHT administration, a blood sample was obtained by heart puncture of three mice in each group at intervals of 6, 12, and 24 h after injection. After clotting overnight, samples were centrifuged at 150,000 × g and the serum was used for analysis. The serum hormone levels were estimated using a radioimmunoassay (9, 10).

Hormone Receptor Assay. An ER assay was performed by using a modification of the method of Tominaga et al. (11), as described previously. In brief, approximately 1 cm³ of the tumors were sonically disrupted in TESH-glycerol buffer at 4°C, followed by centrifuging at 105,000 × g for 60 min at 4°C. The supernatant served as the estrogen receptor fraction. The incubation mixture for the total binding study was composed of 50 μl of TESH-glycerol buffer containing various concentrations of 16α-3H-17β-estradiol and 50 μl of cortisol. After incubation for 18 h at 4°C, 0.5 ml of DCC was added and the preparation was further incubated for 30 min at 4°C, followed by centrifugation at 3,000 × g for 20 min. The supernatant was directly assayed for 3H radioactivity using an Aloka Auto-Gamma counter.

A testosterone receptor assay was done using a variation of the method of Shimazaki et al. (12). The cytosol was incubated in 500 μl of TESH-glycerol buffer containing various concentrations of [1,2,6,7-3H]17β-DHT and 10 mM Na2MoO4 at 4°C for 20 h. After incubation, 0.5 ml of TESH-glycerol buffer containing DCC was added and the preparation was centrifuged. The radioactivity level of the supernatant was determined in 5 ml of toluene (1 liter)/PPO (4 g)/POPOP (0.1 g) in a liquid scintillation counter. In parallel incubations, a 100-fold molar excess of unlabeled ligand was added. Nonspecific binding not displaced under these conditions was subtracted. The dissociation constant (Kd) was determined in 5 ml of toluene (1 liter)/PPO (4 g)/POPOP (0.1 g) in a liquid scintillation counter. In parallel incubations, a 100-fold molar excess of unlabeled ligand was added. Nonspecific binding not displaced under these conditions was subtracted. The dissociation constant (Kd) and binding capacity were determined by Scatchard analysis (13).

Statistical Analysis. Student’s t test was used to determine any statistical differences in tumor growth rates among the various groups.

RESULTS

Effects of Estradiol and Testosterone on in Vitro Cell Growth. Fig. 1 shows the percentage cell growth in KSE-1 cell cultures after treatment of estradiol or DHT relative to cell growth in a control culture. The percentage cell growth in estradiol-treated cultures decreased in a dose-dependent manner (Fig. 1A), reaching 4.0% of the control value at an estradiol concentration of 10³ pg/ml. In DHT-treated cultures, the percentage cell growth increased and reached 145% at a DHT concentration of 10⁻¹ ng/ml (Fig. 1B). On the other hand, the proliferation of KSE-2 cells was not affected by either estradiol or DHT treatment (Fig. 1).

Estrogen and Androgen Receptors. At the 4th week after the implantation of the cells, the developed tumors were assayed for ER and AR. Scatchard analysis revealed that the KSE-1 tumors had a Kd of 0.35 × 10⁻¹⁰ M and a binding content of 4.0 fmol/mg protein for estrogen receptor and a Kd of 0.69 × 10⁻¹¹ M and a binding content of 2.0 fmol/mg protein for DHT receptor whereas neither ER nor AR was detected in the KSE-2 tumors.

Chronological Change of Serum Hormone Levels. The chronological changes in serum hormone levels after the injection of estradiol or DHT are shown in Fig. 2. In the nontreated mice, the serum levels of estradiol and DHT were 49.2 pg/ml and 3.0 ng/ml in males and 63.9 pg/ml and 1.5 ng/ml in females, exhibiting no significant difference between males and females. As shown in Fig. 2A, the administration of estradiol produced an increase in circulating estradiol levels of up to 1000 pg/ml at the 12th h after the first injection in both males and females (approximately 30-fold of the controls). Simultaneously, circulating DHT levels decreased to quantities below the detection level (<0.1 ng/ml) after the second estradiol injection in both sexes.

On the other hand, DHT administration caused serum DHT levels to rise up to 9.1 ng/ml in males (3-fold of the controls) and 11.4 pg/ml in females (8-fold of the controls), as shown in Fig. 2B. Simultaneously, the estradiol levels increased to 410 pg/ml in males and 650 pg/ml in females.

Effects of Hormones on Tumor Growth. Fig. 3 shows the growth rates of KSE-1 tumors possessing both ER and AR. No significant difference was found between males and females. Estradiol administration significantly inhibited the tumor growth in both males and females when compared with non-treated mice (P<0.01). DHT administration did not significantly alter the tumor growth rates of KSE-1 cells in either sex.

As shown in Fig. 4, the growth rates of KSE-2 tumors with neither ER nor AR were unaffected by either estradiol or DHT injections in either sex.

DISCUSSION

Sex hormone receptors have been found in malignant tumors of human digestive organs such as the stomach (14), colon (15), and liver (16), as well as hormone-sensitive organs like the breast (17), uterus (18), and prostate (19). In esophageal cancer, however, there is little documentation on the presence of hormone receptors (20, 21). Since our report about a KSE-1 cell line exhibiting binding contents for both ER and AR in culture (7), we have found one more clinical case of ER-positive esophageal carcinoma (22). These findings suggest the possibility that some esophageal carcinomas may have hormone receptors and that their cell growth might be modulated by administering sex hormones.

The present study demonstrated that the in vivo growth of an esophageal carcinoma cell line (KSE-1) possessing hormone receptors was responsive to hormonal manipulation by the systemic administration of sex hormones. Estradiol administration exerted a potent inhibitory effect on the growth of KSE-1 tumors in which the serum estradiol level increased and the serum DHT level decreased concomitantly. The decrease in the growth rates of the tumors in the estradiol-treated mice seems to have been caused by the following: increased growth suppression effects due to an increased estradiol level in the serum; the lack of growth stimulation due to a decrease in the DHT serum level. However, DHT administration did not elevate the growth
rates of KSE-1 tumors, although the serum levels of DHT increased significantly (i.e., 3-8 fold of nontreated mice). This may be due to the fact that intact rather than castrated mice were used and consequently the circulating estradiol even in the DHT-treated mice consistently inhibited the tumor growth. There was also no significant difference in the tumor growth rates of KSE-1 cells between male and female mice. This lack of sexual difference in tumor growth could be reflected by the similar levels of circulating estradiol and DHT in both sexes.

The present experiment indicated that cell growth of KSE-1 esophageal carcinoma cells was inhibited by estradiol treatment. In addition, we have reported that the primary cultured cells taken from a clinical case of esophageal carcinoma with ER revealed the inhibitory growth-regulatory effects of estradiol (22). Similar phenomena have also been observed in malignant tumors in other human organs. For example, Bronzert et al. (23) isolated an estrogen-inhibited cell line from MCF-7 breast cancer cells. Cell proliferation of this variant cell line possessing ER with a level similar to that of the wild MCF-7 cell line was inhibited by estradiol, while the wild type MCF-7 cell line was stimulated by estradiol (24). The growth of ER-positive human colorectal (15) and gastric carcinoma (25) xenografted into nude mice was inhibited by estradiol administration. Although the mechanism of the inhibitory effects of estradiol on the growth of esophageal carcinoma cells has not yet been clarified, we have noted that KSE-1 cells and other primary cultured cells of esophageal carcinomas possess a larger number of binding sites for estradiol in the nuclei than those in the cytosol (26).

The inhibitory effects of the systemic administration of estradiol on the in vivo growth of esophageal carcinoma might give a clue to the clinical use of hormone therapy in the treatment of esophageal carcinoma, without the necessity of ablative surgery such as castration. At present, however, there are still complex problems with regard to hormone therapy in the treatment of esophageal carcinoma. Not all esophageal carcinomas have sex hormone receptors and the cell growth of

![Fig. 2.](image1) Chronological changes of the circulating sex hormone levels in the estradiol (E$_2$)-treated (A) or DHT-treated (B) mice. After i.p. injection of 10$^{-3}$ mg estradiol or 10$^{-4}$ mg DHT, serum concentrations of estradiol and DHT were measured in males (-----) and females (-----) at the times indicated. In estradiol-treated mice, an increase in the estradiol level and a simultaneous decrease in the DHT level were observed (A). In DHT-treated mice, an increase in the serum levels of both DHT and estradiol was observed (B). Bars, SD in the five mice. * below the detection level.

![Fig. 3.](image2) Effects of the administration of sex hormones on the growth of KSE-1 tumors in nude mice. In both males (A) and females (B), a significant growth inhibition of KSE-1 tumors was observed in the E$_2$-treated mice (○) compared with the control mice (●) (*, P < 0.01; **, P < 0.05). There was no significant difference in the growth rates between the DHT-treated mice (△) and the control mice. Bars, SD in the five mice.
carnicomas containing estrogen-binding components is not always inhibited/enhanced by estradiol. One solution to these problems is to establish an assay system to determine whether estradiol enhances or inhibits the growth of tumor cells. With this in mind, we have recently developed an in vitro assay system for predicting estradiol sensitivity of clinical cancer cells (22).

Further investigation is needed to better elucidate the biological mechanisms in which sex hormones regulate the growth of esophageal carcinoma cells, such as defining the relevance of intermediate polypeptide growth factors (27). KSE-1 cells containing both ER and AR may provide useful in vitro and in vivo systems for the analysis of the sex hormones on the growth of esophageal carcinoma.

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REFERENCES

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