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Interrelationship between Estradiol and Tamoxifen Responses for Clinical Breast Carcinoma Cells Cultured on Contact-sensitive Plates

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An *in vitro* assay system for predicting the estradiol (E_2) sensitivity of clinical cancer cells was applied to 54 patients with breast carcinoma to compare the responses to E_2 and tamoxifen (TAM) with the estrogen receptor (ER) status. We found that 18 of the 35 cases in the ER-positive group and 6 of the 19 cases in the ER-negative group were stimulated by E_2. It is suggested that ER status alone can not predict the response of cultured cells to E_2 in clinical breast cancer. Cell growth of 11/35 (31%) of the ER-positive cases and that of 8/19 (42%) of the ER-negative cases was inhibited by E_2. Since the cases inhibited by E_2 could not be distinguished by ER status alone, an assay system based on a quantitative proliferative response was considered necessary. There were 20 (83%) cases of inhibition by TAM among the 24 stimulated by E_2. Only 18/35 (51%) of the ER-positive group exhibited growth inhibition by TAM. In our (CSP) assay, 20 (83%) of the 24 cases stimulated by E_2 were inhibited by TAM, 10 (91%) of the 11 E_2-insensitive cases were insensitive to TAM and 13 (68%) of the 19 cases inhibited by E_2 were stimulated by TAM. In short, TAM response and E_2 response tended to be inversely related (43/54=80%, $P<0.01$). Furthermore, the E_2-response rate showed a good correlation with the TAM-response rate ($R^2=0.825$). These results indicate the feasibility of predicting individual tumor responses to either E_2 or TAM by using CSPs.

Key words: Breast cancer — Estradiol — Tamoxifen

The determination of ER[5] content from human tumor specimens helps in the establishment of hormone therapy and has a predictive value for the course of the disease,[1] especially in breast cancer. However, the imperfect correlation between ER content and sensitivity to the therapeutic anti-estrogen TAM[2] has spurred many laboratories to seek complementary tests capable of improving the detection of anti-estrogen sensitivity.[3–6] It has been shown that cell growth of a variant MCF-7 line possessing ER at a similar level to the wild MCF-7 cell line[7] was inhibited by estradiol E_2, while the wild-type MCF-7 cell line[8] was stimulated by E_2. We also demonstrated that the proliferation of an esophageal cancer cell line (KSE-1) having ER was inhibited by E_2 treatment *in vitro*.[9] It is thus probable that E_2 does not always stimulate cell growth of cancer cells in the clinical context.

Therefore, we developed an E_2 sensitivity test to determine whether E_2 stimulates or inhibits the proliferation of cancer cells.[10] This sensitivity test predicts the E_2 response of clinical cancer cells by using CSPs of confluent BALB/c 3T3 cell monolayers, on which neoplastic cells are capable of growing while contact-sensitive normal cells are not.[11] This assay system, which is based on the quantitative proliferative response, can provide information, not available by a study of ER status alone, on the role of E_2 and TAM in the proliferation of clinical breast cancer cells.

MATERIALS AND METHODS

Chemicals EMEM, without phenol red,[12] was purchased from Nissui pharmaceutical Co., Ltd. FBS was obtained from M. A. Bioproducts. Gentamicin was supplied by Schering Co. Amphotericin B was from Toyama Chemical Co., Ltd. E_2 (17β-estradiol) and TAM were purchased from the Sigma Chemical Co. They were dissolved in ethanol (final ethanol concentration, $<0.1\%$) and then directly diluted to the desired concentration with a fresh medium containing 2.5% FBS. The latter was prepared with dextran-coated charcoal (DCC; 5% charcoal, 0.5% dextran) and was essentially free of E_2.[13] The activated charcoal powder was obtained from Wako Pure Chemical Industries, Ltd. Dextran T-70 was obtained from Pharmacia. 16α-^{125}I-17β-Estradiol and [^{3}H]dThd were purchased from Amersham.

Cell culture BALB/c 3T3 A-31 cells, originally isolated by Kakunaga,[14] were recloned in our laboratory. The

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[5] The abbreviations used are: ER, estrogen receptor; CSPs, contact-sensitive plates; EMEM, Eagle's minimal essential medium; FBS, fetal bovine serum; DCC, dextran-coated charcoal; E_2, estradiol; TAM, tamoxifen; [^{3}H]dThd, [^{3}H]thymidine.

MCF-7 cell line[15, 16] was obtained from the Japanese Cancer Research Resources Bank.

Preparation of CSPs Confluent monolayers were prepared by inoculating 3×10^4 BALB/c 3T3 cells into 35-mm tissue culture dishes (Corning 2500). The medium was changed at confluence, and the plates were incubated for an additional 2 days to ensure complete confluence [1.45 ± 0.23(SD)$\times10^5$ cells/dish]. The confluent monolayers of these contact-sensitive cells were termed CSPs.[17] The cells on the confluent monolayers which were to be tested, were superinoculated with a fresh medium.

Cell growth on CSPs The cell growth of normal and neoplastic cells on CSPs and plastic dishes was evaluated by measuring the amount of [^{3}H]dThd incorporation into DNA. The tissue culture conditions were determined according to the method of Aitken and Lippman.[18]

Approximately 10^4 cells of MCF-7 or 5×10^4 cells of BALB/c 3T3 per 35-mm dish at the logarithmic growth phase were cultured on CSPs. It was previously demonstrated that CSPs retained the characteristic of contact sensitivity after treatment with E_2 and TAM at concentrations of $<10^2$ ng/ml and $<10^1$ ng/ml, respectively.[19] The medium was replaced with EMEM/DCC containing 10^{-5} mol of phosphate/liter but no asparagine. According to the growth pattern of MCF-7 cells, the cultures were then either further incubated with 10^{-2} ng/ml E_2 or 10^1 ng/ml TAM, or had no further treatment for 24–96 h.

Patients Sixty-nine women with breast carcinoma were admitted to the Department of Surgery, Oita Prefectural Hospital, from 1990 to 1992. All of these patients underwent surgery. After surgery, a sensitivity test to either E_2 or TAM was performed on a part of the primary lesion. An ER assay was also done on another portion. A statistically significant increase of cancer cell growth in nontreated culture from the 48th to 120th h of primary culture on CSPs was observed in 54 of 69 cases (78.3%).[10] These 54 cases were considered to be evaluable for growth response to E_2 and TAM. The number of these patients in each clinical stage was 11 in stage I, 38 in stage II, and 5 in stage III. The histopathological types of these patients were invasive ductal carcinoma (35 cases), medullary carcinoma (10 cases), scirrhous carcinoma (13 cases), and lobular carcinoma (1 case).

E_2 and TAM sensitivity test for clinical carcinomas Clinical carcinoma tissue specimens were minced with trimming blades measuring <1 mm^3. A minced tumor specimen was then soaked for 4 min in 10% EMEM consisting of 100 μg/ml of sodium piperacillin, 5 μg/ml of gentamicin, and 5 μg/ml of amphotericin B. This was repeated 5–7 times with renewals of the antibiotic solution. The tumor fragments were then placed in a flask containing 10% EMEM and 0.14% collagenase type 1 and stirred for 30 min at 37°C to allow enzymatic digestion. A single suspension of tumor cells was prepared by the mechanical pipeting of the digested specimen into a fresh medium. After counting of the number of viable cells in a 0.4% trypan blue solution by using a hemocytometer, these tumor cells were used for the experiment. The primary tumor cells were superinoculated onto CSPs at a density of approximately 10^4 cells/35-mm dish. After a 48-h incubation, the cells were treated with either E_2 (10^{-2}–10^{-1} ng/ml) or TAM (10^{-1}–10^{-2} ng/ml) for 48 h in EMEM/DCC.

Quantitative measurement of cell growth The cell number per dish was counted by a Coulter counter (Coulter Electronics, Inc.) immediately after the E_2 or TAM treatment. To determine [^{3}H]dThd incorporation into DNA, the cultures were labeled with 0.5 μCi of [^{3}H]dThd/ml for 8 h. The cells were washed five times with PBS and then trypsinized. The trypsinized cells were removed from the plates by repeated pipeting and were then sonically disrupted and treated with trichloroacetic acid (TCA) at 3000g for 20 min. The pellets were dissolved in 0.3 N KOH and maintained at 37 °C for 20 h. The radioactivity of [^{3}H]dThd incorporated into the TCA-insoluble materials was determined by using a scintillator (ACS-II).

Determination of cell growth and response to either E_2 or TAM The percentage cell growth in either E_2- or TAM-treated cultures was determined by use of the following formula;

$$\text{Percentage cell growth (\%)} = (A-C)/(B-C)\times100$$

in which A = [^{3}H]dThd incorporation into the E_2- or TAM-treated culture, B = [^{3}H]dThd incorporation into the nontreated culture, and C = [^{3}H]dThd incorporation into the background of CSP alone. The following criteria were used to evaluate the response to E_2: (i) stimulated by E_2 = >110% cell growth, (ii) insensitive to E_2 = 90–110% cell growth, (iii) inhibited by E_2 = <90% cell growth. When the percentage of cell growth was over 110% or under 90%, the difference of the growth between the treated and control cultures was statistically significant ($P<0.05$).

The response rate of the cancer cells treated with TAM was determined by use of the following formula;

$$\text{TAM-response rate (\%)} = (\text{\% cell growth in } E_2\text{-treated cultures}) - (\text{\% cell growth in TAM-treated cultures})$$

The following criteria were used to evaluate TAM response: (i) inhibited by TAM = >10% difference in cell growth between E_2 and TAM treatments,(ii) insensitive to TAM = difference of between −10% and 10%, (iii) stimulated by TAM = <−10% difference in cell growth between E_2 and TAM treatments.

ER assay The ER assay was performed by a modification of the method of Tominaga *et al.*[9] In brief, approxi-

mately 1 cm^3 of clinical carcinoma specimen was sonically disrupted in 0.02 *M* Tris-HCl-0.0015 *M* EDTA-0.012 *M* monothioglycerol-5% glycerol, pH 7.8, at 4°C, followed by centrifugation at 105,000*g* for 60 min at 4°C. The supernatant served as the ER fraction. The incubation mixture for the total binding study was composed of 50 μl of TESH-glycerol buffer (0.02 *M* Tris-HCl-0.0015 *M* EDTA-0.012 *M* monothioglycerol-5% glycerol, pH 7.8) containing various concentrations of 16α-^{125}I-17β-E_2 and 50 μl of cytosol. The supernatant was directly assayed for ^{125}I radioactivity, using an Aloka Auto-Gamma counter. Since values of 7 fmol/mg or more of cytosol protein are measurable, a result such as this should rule out any artifacts resulting from inadequate methodology.[20]

Statistical analysis The chi-square test was used to determine the statistical significance of differences between the various groups. The correlation between E_2 and TAM response of cultured cancer cells was analyzed by the method of Pearson.

RESULTS

Cell proliferation and response to either E_2 or TAM of MCF-7 cells on CSPs As shown in Fig. 1A, the growth of MCF-7 cells treated with E_2 or TAM was approximately 208% or 73% of the untreated control, respectively, at 72 h (significantly different from the nontreated culture). Therefore 72-h incubation of the primary culture cells with E_2 or TAM was chosen as the standard condition. Fig. 1B shows the incorporation of [^{3}H]dThd into DNA of MCF-7 cells treated with either E_2 or TAM for 72 h beforehand. On the basis of this result, the labeling time of primary culture cells with [^{3}H]dThd was set at 10 h.

Influence of TAM on contact sensitivity Table I shows the change in contact inhibition of the CSPs after TAM treatment for 72 h. [^{3}H]dThd was only slightly incorporated into the CSPs in the medium with both 10% EMEM and 10% EMEM/DCC. When even 5×10^4 BALB/c 3T3 cells (a large number of contact-sensitive cells) were superinoculated onto the CSPs in 10% EMEM/DCC and incubated for 72 h, the [^{3}H]dThd incorporation was 420 cpm, almost equal to the [^{3}H]dThd incorporation of the CSP alone. The [^{3}H]dThd incorporation of CPSs treated with TAM was nearly equal to that of CSPs without treatment. The contact sensitivity of CPSs was not changed by E_2 or TAM at the concentration of this experiment.

Comparative study of ER status and E_2 response As shown in Table II, in our 54 cases, 35 (65%) were ER-positive cases (>7 fmol/mg protein) in the chemical ER assay and 24 (44%) were E_2-stimulated cases (>110%) in the CSP assay. There were 29 (83%) E_2-sensitive cases in the ER-positive group and 14 (74%) E_2-sensitive cases in the ER-negative group, with no statistically significant difference ($P>0.1$). In the 35 ER-positive cases, there were 18 (51%) E_2-stimulated cases and 17 (49%) E_2-unstimulated cases. The proliferation of primary cultured cells in nineteen (35%) of the 54 cases was inhibited by E_2. Of these nineteen cases, eleven (58%) possessed ER. In the ER-negative cases, six (32%) were stimulated by E_2 and eight (42%) were inhibited by E_2.

Comparative study of ER status and TAM response As shown in Table III, primary breast cancer cell growth of

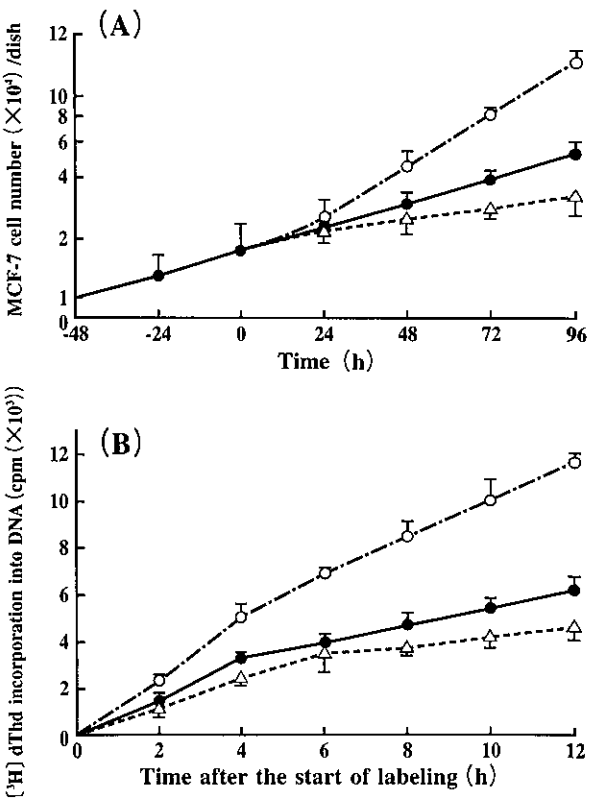

Fig. 1. Cell growth curve (A) and [^{3}H]dThd incorporation into DNA (B) of MCF-7 treated with E_2 or TAM. In A, MCF-7 cells (10^4) were superinoculated onto CSPs and incubated for 48 h. Thereafter, the cultures were incubated with either 10^{-1} ng/ml E_2 (○) or 10^0 ng/ml TAM (△), or neither (●). The cell number in each dish was counted with a Coulter counter at the times indicated. In B, MCF-7 cells, superinoculated onto CSPs, were further incubated with 10^{-1} ng/ml E_2(○) or 10^0 ng/ml TAM (△), or neither (●) for 72 h and then incubated with 0.5 μCi/ml of [^{3}H]dThd (time 0). Cells were harvested at the times indicated. Cell growth (cell number or [^{3}H]dThd incorporation into DNA) = A−B, in which A = MCF-7 cell cultures on CSPs and B = CSPs alone; bars, SD for three dishes.

22 (41%) of all 54 cases was inhibited by TAM treatment. In the ER-positive group, 18 cases (51%) were inhibited by TAM. In the ER-negative group, cell growth of four cases (21%) was inhibited by TAM. The difference in inhibitory response to TAM between the ER-positive and ER-negative groups was statistically significant ($P=0.03$). Of the 22 TAM-inhibited cases, 18 (90%) were ER-positive. Of all 54 cases studied, the cell growth of 14 (26%) was stimulated by TAM. There were 27 (77%) and 9 (47%) TAM-sensitive (stimulated or inhibited) cases in the ER-positive and in the ER-negative groups, respectively, and the difference was statistically significant ($P<0.05$).

Comparative study of responses to E_2 and TAM As shown in Table IV, in 20 (83%) of the 24 E_2-stimulated cases, cell growth was inhibited by TAM. There were 20 (91%) E_2-stimulated cases among the 22 TAM-inhibited cases, and 13 (93%) E_2-inhibited cases among the 14 TAM-stimulated cases. The cell growth of 10/11 (91%) of the E_2-insensitive group was insensitive to TAM. The cell growth of 13/19 (68%) of the E_2-inhibited group was stimulated by TAM. TAM sensitivity showed a close relation to E_2 sensitivity ($P<0.01$). As shown in Fig. 2, a statistical analysis, using Pearson's method, indicated a good correlation between the relative responses to E_2 and TAM treatment ($y=-75.2+0.85x$, $R^2=0.825$).

Comparative study of ER status, and responses to E_2 and TAM As shown in Table V, in the ER-positive group, cell growth responses to TAM were as follows: (i) inhibition was observed in 16/18 (89%) of the cases which gave a stimulatory response to E_2, (ii) TAM-insensitivity was observed in 5/6 (83%) of the E_2-insensitive cases, and (iii) stimulation was observed in 8/11 (73%) of the cases which showed an inhibitory response to E_2.

In the ER-negative group, cell growth response to TAM was as follows: (i) inhibition was observed in 4/6 (67%) cases stimulated by E_2, (ii) TAM-insensitivity was observed in 5/5 (100%) of E_2-insensitive cases, and

Table I. [^{3}H]dThd Incorporation into Normal BALB/c 3T3 Cells onto Preformed CSPs

Medium	[^{3}H]dThd incorporation per 35-mm dish	
	CSP alone	3T3 cells on CSP
EMEM	410±0	380±90
EMEM/DCC	490±0	420±10
EMEM/DCC+10^{-1} ng/ml E_2	460±20	450±90
EMEM/DCC+10^{-2} ng/ml TAM	390±70	430±100
EMEM/DCC+10^{-1} ng/ml TAM	470±60	410±40
EMEM/DCC+10^{0} ng/ml TAM	390±80	420±60
EMEM/DCC+10^{1} ng/ml TAM	440±30	460±70

Normal BALB/c 3T3 cells (5×10^4) were co-cultivated on CSPs treated with drugs at various concentrations for 72 h. The cultures were labeled with 0.5 μCi/ml of [^{3}H]dThd for 10 h. Each value represents the mean±SD for triplicate plates.

Table II. Comparative Study: ER Status and Response to E_2

ER status[a]	No. of cases	Response to E_2		
		Stimulated	Insensitive	Inhibited
(+)	35	18 (51%)	6 (17%)	11 (31%)
(−)	19	6 (32%)	5 (26%)	8 (42%)
Total	54	24 (44%)	11 (20%)	19 (35%)

a) ER status: ER(+) >7 fmol/mg protein, ER(−) <7 fmol/mg protein.

The response rate of cancer cells to E_2 was determined by applying the following formula: cell growth (%) = (A−C)/(B−C)×100, in which A = E_2-treated culture, B = nontreated culture, and C = background of CSP alone. The following criteria were used for E_2 response: (i) stimulated by E_2 = >110% cell growth, (ii) insensitive to E_2 = 90–110% cell growth, (iii) inhibited by E_2 = <90% cell growth.

Table III. Comparative Study: ER Status and Response to TAM

ER status	No. of cases	Response to TAM		
		Stimulated	Insensitive	Inhibited
(+)	35	9 (26%)	8 (23%)	18 (51%)
(−)	19	5 (26%)	10 (53%)	4 (21%)
Total	54	14 (26%)	18 (33%)	22 (41%)

The response rate of the cancer cells to TAM was determined by applying the following formula: TAM response = (% cell growth after E_2 treatment) − (% cell growth after TAM treatment). The following criteria were used for TAM response: (i) inhibited by TAM = >10% difference of cell growth between E_2 and TAM treatments, (ii) insensitive response = difference of between −10% and 10%, (iii) stimulated by TAM = <−10% difference in cell growth between E_2 and TAM treatments.

Table IV. Comparative Study: Responses to E_2 and TAM

Response to E_2	No. of cases	Responses to TAM		
		Stimulated	Insensitive	Inhibited
Stimulated	24	1 (4%)	3 (13%)	20 (83%)
Insensitive	11	0	10 (91%)	1 (9%)
Inhibited	19	13 (68%)	5 (26%)	1 (5%)
Total	54	14 (26%)	18 (33%)	22 (41%)

Table V. Comparative Study: ER Status and Responses to E_2 and TAM

ER status	Responses to E_2	Response to TAM		
		Stimulated	Insensitive	Inhibited
ER(+)	Stimulated (18)	1	1	16
	Insensitive (6)	0	5	1
	Inhibited (11)	8	2	1
ER(−)	Stimulated (6)	0	2	4
	Insensitive (5)	0	5	0
	Inhibited (8)	5	3	0
Total	54	14	18	22

(iii) stimulation was observed in 5/8 (63%) cases inhibited by E_2. TAM sensitivity showed a close relation to E_2 sensitivity in both ER-positive and ER-negative groups.

DISCUSSION

The influence of E_2 on the multiplication of clinical target cells remains unclear. We have therefore developed an *in vitro* assay system to predict the E_2 sensitivity of clinical carcinoma cells by measuring the effects of E_2 on the DNA synthesis of primary culture cells.[10] The superinoculation of neoplastic and normal cells onto confluent monolayers of a contact-sensitive cell line, BALB/c 3T3 A-31[3] (designated as contact-sensitive plates, CSPs), resulted in both the specific growth of the neoplastic cells and the growth inhibition of contact-sensitive normal cells. Therefore, when a cell suspension obtained from a clinical tumor, presumably including neoplastic and normal cells, is cultivated on CSPs, the growth of neoplastic cells can be specifically examined by measuring DNA synthesis.[10, 19] In this report, we compared both E_2 sensitivity and TAM sensitivity obtained by our CSP assay with the ER status obtained by a chemical assay of clinical breast cancer specimens.

Firstly, we compared ER status and E_2 sensitivity found in the CSP assay. In the ER-positive group, there were 29 (83%) E_2-sensitive cases. However, in the ER-positive group, there were only 18 (51%) E_2-stimulated cases, while cell growth of 11 (31%) cases was inhibited by E_2. This is inconsistent with the general view that the growth of ER-positive cases is stimulated by E_2 while that of ER-negative cases is insensitive to E_2.[21] In this study, such results were obtained in only 23 (43%) of all 54 cases. However, statistical analysis indicated a good correlation between ER status and the relative increase of [^{3}H]TdR incorporation into DNA of both E_2-stimulated and -insensitive cases (R^2=0.763). A similar result has been previously reported by us.[10]

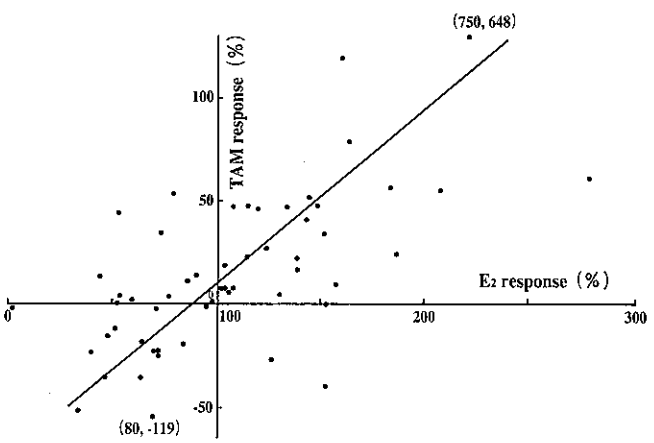

Fig. 2. Statistical analysis of the correlation between E_2 and the TAM response rate of primary cultured cancer cells. The correlation coefficient was established according to the method of Pearson.

In this study, there were 19 (35%) E_2-inhibited cases among the 54 clinical breast carcinomas. This phenomenon of growth inhibition by E_2 has also been observed in various neoplastic cell lines. For example, Bronzert *et al.* isolated an E_2-inhibited cell line from MCF-7 breast cancer cells.[7] Cell proliferation of this variant cell line with ER at a similar level to that of the wild-type MCF-7 cell line was inhibited by E_2, while the wild-type MCF-7 cell line was stimulated by E_2.[15] The growth of human carcinoma cell lines derived from other organs, such as the esophagus,[9] colon,[21] and stomach[22] has also been reported to be inhibited by a physiological level of E_2.

In the ER-negative group, cell growth of six (32%) ceses was stimulated by E_2, and that of eight (42%) cases was inhibited by E_2. Primary cancer cells showing stimulative and inhibitive responses have already been reported.[10] It has been hypothesized that E_2 induces the synthesis and/or release of a specific growth factor of E_2-sensitive cells in an autocrine manner.[23] The inhibiting effect of E_2 on the proliferation of its target cells may thus be considered as being due to a direct action of E_2 on the cell through a mechanism independent of the ER.

Secondly, we compared ER status and TAM response obtained in our CSP assay. The rate of TAM-inhibited cases in the ER-positive group (18/35=51%) was higher than that in the ER-negative group (4/19=21%). There were 18 ER-positive cases in the TAM-inhibited group (18/22, 82%). In other words, the success rate of TAM treatment in the ER-positive group would be higher than that in the ER-negative group, as has been reported by several researchers.[1, 24] Therapy with the antiestrogen TAM is a typical example of endocrine treatment for breast cancer which has been used over the last two decades. The exact mechanisms of TAM action, however, have not yet been fully elucidated. ER is thought to be the main target of antiestrogen because a close correlation exists between the presence or absence of ER in breast cancer and the response of breast cancer to endocrine therapy.[1, 24] However, ER status alone appears to be unable to account fully for clinical breast carcinoma response to TAM.

Thirdly, we compared E_2 response with TAM response. The E_2 response showed a close inverse relation to the TAM response. Of all 54 breast carcinomas, 43 (80%) cases showed an inverse relationship between responses to TAM and E_2, i.e. there were: (i) 20 (91%) E_2-stimulated cases in the 22 TAM-inhibited cases, (ii) 10 (91%) TAM-insensitive cases in the 11 E_2-insensitive cases, and (iii) 13 (93%) E_2-inhibited cases in the 14 TAM-stimulated cases. Moreover, the response rate to TAM correlated closely with that to E_2 ($R^2=0.825$). These results indicate that TAM antagonizes the action of E_2, i.e., TAM reverses the effect of E_2 on E_2-sensitive cancer cells. It has been generally recognized that the E_2-receptor complex, whereby E_2 enters the cell and binds to a specific receptor protein, stimulates DNA replication in the nucleus and then initiates or accelerates a specific RNA synthesis. Messenger RNA generates the corresponding protein, which participates in physiological cell function. In addition, E_2 induces the synthesis and/or a release of growth factors (estromedins)[25] or facilitating factors (plasminogen activator).[26] Cell proliferative functions are also regulated by enzymes, such as thymidine kinase, DNA polymerase, ornithine decarboxylase, etc.[17, 27, 28] Thus, we suggest that TAM blocks, not only the E_2 binding receptor, but also some other site(s) concerned with the proliferative function of E_2 for primary cultured cells of clinical breast carcinoma and cancer cell lines.

Our results suggest that it is feasible to predict individual response by determining whether the growth of clinical breast cancer cells treated with either E_2 or TAM is inhibited or stimulated. Our assay could be a useful indicator in reaching a decision as to the advisability of TAM treatment for breast cancer patients.

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