[CANCER RESEARCH 51, 5951-5955, November 1, 1991]

# Involvement of Transforming Growth Factor α/Epidermal Growth Factor Receptor Autocrine Growth Mechanism in an Ovarian Cancer Cell Line *in Vitro*

**Ken-ichiro Morishige, Hirohisa Kurachi,[1] Kyoka Amemiya, Hiroshi Adachi, Masaki Inoue, Akira Miyake, Osamu Tanizawa, and Yasuhiko Sakoyama**

*Departments of Obstetrics and Gynecology [K-i. M., H. K., K. A., H. A., M. I., A. M., O. T.] and Genetics [Y. S.], Osaka University Medical School 1-1-50 Fukushima, Fukushima-ku, Osaka 553 Japan*

## ABSTRACT

Although transforming growth factor (TGF) α and epidermal growth factor (EGF) receptor (EGFR) autocrine mechanism is widely demonstrated in many kinds of cancers, its biological significances still remain circumstantial. We critically assessed the significance of this mechanism on the growth of an ovarian cancer cell line. Northern blot analysis in polyadenylated RNA isolated from cells by using $^{32}$P-labeled pre-TGFα, EGFR, and prepro-EGF complementary DNAs as probes revealed that pre-TGFα and EGFR but not prepro-EGF gene transcripts were expressed in the cell. TGFα and EGFR but not EGF proteins were observed by immunocytochemical stainings, using monoclonal antibodies against human TGFα, EGFR, and EGF, respectively. This cell line possessed a class of high affinity EGF receptor by $^{125}$I-EGF binding studies; $K_d$ being $2.9 \times 10^{-10}$ M and $B_{max}$ to be $7.7 \times 10^4$ sites/cell. As much as 1.12 ± 0.14 ng (SD; $n = 3$)/10$^7$ cells/24 h of TGFα was secreted in the conditioned media. These results suggested the expression of a TGFα/EGFR autocrine mechanism in this cell line. We, therefore, assessed the biological significance of this mechanism on the growth of this cell line in serum-free monolayer cell cultures. Although 0.1, 1.0, and 10 nM concentrations of TGFα did not show significant growth promotion, monoclonal antibodies against TGFα and EGFR but not EGF significantly inhibited cell growth. All these data suggested the biological importance of a TGFα/EGFR autocrine mechanism on the growth of this cell line *in vitro*.

## INTRODUCTION

Autocrine mechanisms are believed to play a crucial role in the growth in many kinds of cancers (1-4) and cancer cell lines (5-7). Various autocrine mechanisms by TGFα[2] (1-6), bombesin (7), and insulin-like growth factor (6) are known to be expressed in cancer cells. Among these, an autocrine growth mechanism by bombesin in human small cell lung cancer is well established (8).

It is well known that TGFα shares a common receptor with EGF (9). Although the TGFα/EGFR autocrine mechanism is most commonly demonstrated in various cancers (1-4) and cancer cell lines (5, 10), its biological significances on the growth of cancer cells still remain to be clarified.

Growth mechanisms of ovarian cancers are not well known. Elevated levels of TGFα are reported in the urine (11) and in the ascites fluid (12) from patients with disseminated ovarian cancers, and EGFR is rather commonly expressed in primary ovarian cancers (13). These findings suggest the possible presence of a TGFα/EGFR autocrine mechanism in human ovarian cancers. We, therefore, studied the expression of TGFα and EGFR and the biological significance of the TGFα/EGFR mechanism on cell growth *in vitro* in a human ovarian serous cystadenocarcinoma derived cell line, designated SHIN-3 (14).

## MATERIALS AND METHODS

**Materials and Cell Lines.** Materials were purchased as follows: Na$^{125}$I was from New England Nuclear (Boston, MA), [α-$^{32}$P]dCTP and Multiprime DNA labeling system were from Amersham Laboratories (Buckinghamshire, England), anti-human TGFα (antibody 1) and anti-EGFR mAbs [antibody 1 which is known to inhibit EGF binding (15) and antibody 2 which does not inhibit EGF bindings (15)] were from Oncogene Science, Inc. (Manhasset, NY), anti-human EGF mAb was from Wakunaga Pharmaceutical Co. (Hiroshima, Japan). All mAbs containing preservations (0.1% sodium azide and others) were extensively dialyzed against 0.01 M PBS, pH 7.4, at 4°C for 48 h and were used in cell cultures. Human prepro-EGF cDNA was a kind gift from Dr. G. I. Bell (University of Chicago, Chicago, IL), human pre-TGFα cDNA, and pE7, a cDNA clone of human EGFR, were generously supplied by Dr. T. Yamamoto (Institute of Medical Science, University of Tokyo, Tokyo, Japan). Anti-CA-125 mAb and ELISA kit for TGFα assay (16) were kindly supplied by Dainabot Co., Ltd. (Tokyo, Japan) and by Dr. Shigeaki Tanaka, Hoechst Japan Limited (Saitama, Japan), respectively.

An ovarian cancer cell line derived from CA-125 producing human serous cystadenocarcinoma (SHIN-3) was used in all the studies. Cells were maintained in RPMI 1640 culture medium (GIBCO, Grand Island, NY) supplemented with 10% FBS (Cell Culture Laboratories, Cleveland, OH) at 37°C in a humidified 95% air, 5% $CO_2$ atmosphere.

**Northern Blot Analysis.** Poly(A)$^+$ RNA was selected by using oligodeoxythymidylate cellulose column (17) after isolating approximately 2 mg of total cellular RNA by the guanidine cesium chloride method (18) from 10$^8$ SHIN-3 cells. Two μg of poly(A)$^+$ RNA was fractionated in 1% agarose/0.66 M formaldehyde gel and transferred onto a nitrocellulose filter according to the standard method (19). Filters were baked at 80°C for 2 h, and were prehybridized and hybridized with $^{32}$P-Multiprime-labeled pre-TGFα (specific activity, $1 \times 10^9$ cpm/μg), prepro-EGF (specific activity, $1 \times 10^9$ cpm/μg), and EGFR (specific activity, $1.5 \times 10^9$ cpm/μg) cDNA probes at 42°C for 24 h in a hybridizing buffer containing 50% formamide, 5× SSC, 5× Denhardt's solution (1× Dehardt's, 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% BSA), 50 mM sodium phosphate, pH 7.0, 0.1% sodium dodecyl sulfate, and 250 μg/ml salmon sperm DNA, as described by Wahl *et al.* (20). Then the filters were washed 3 times with 2× SSC, 0.1% sodium dodecyl sulfate at room temperature for 5 min and twice with 0.3× SSC, 0.1% sodium dodecyl sulfate at 55°C for 30 min, and autoradiographed at −80°C for 2-5 days with an intensifying screen. Probes were removed by immersing the filters in 10 mM sodium phosphate buffer containing 50% formamide at 65°C for 1 h, and rehybridized with β-actin.

**Immunocytochemistry.** SHIN-3 cells were attached to 8-chamber slides (Nunc, Inc., Naperville, IL). Cells were rinsed and fixed with 4% paraformaldehyde, 0.5% glutaraldehyde, and 0.2% picric acid in 0.1 M PBS, and immunocytochemical staining was performed by using the Vectastain ABC kit (Vector Laboratories, Burlingame, CA) according to the recommendations of the manufacturer. mAbs against TGFα (1:100 dilution), EGF (1:100), EGFR (1:100), and CA-125 (1:500) were

Received 5/28/91; accepted 8/27/91.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] To whom requests for reprints should be addressed, at Department of Obstetrics and Gynecology, Osaka University Medical School, 1-1-50 Fukushima, Fukushima-ku, Osaka 553 Japan.

[2] The abbreviations used are: TGFα, transforming growth factor α; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; mAb(s), monoclonal antibody(ies); cDNA, complementary DNA; ELISA, enzyme-linked immunosorbent assay; poly(A)$^+$RNA, polyadenylated RNA; FBS, fetal bovine serum; PBS, phosphate-buffered saline; BSA, bovine serum albumin; 1× SSC, 0.15 M NaCl and 0.015 M sodium citrate.



Downloaded from http://aacrjournals.org/cancerres/article-pdf/51/21/5951/2445250/cr0510215951.pdf by guest on 25 March 2023

used as primary antibodies. Nonimmune mouse IgG, at the same concentrations, were used as negative controls.

**$^{125}$I-EGF Binding Studies.** Cells were grown to subconfluence in RPMI 1640 medium with 10% FBS in 24-well plates (Falcon, Becton Dickinson, Lincoln Park, NJ) and $^{125}$I-EGF binding studies were performed by adding 0.01–100 pmol of $^{125}$I-EGF (specific activity, 150–200 $\mu$Ci/$\mu$g) with or without excess amounts of unlabeled EGF to the medium. Cells were incubated at 20°C for 3 h, washed 3 times with chilled Hanks' solution with 0.1% BSA, and the radioactivities on the cell were counted by a gamma counter (Minaxi $\gamma$, Packard Instrument Co., Meriden, CT). All assays were carried out in triplicate, and the specific binding data were plotted according to the method of Scatchard.

**Preparation of Conditioned Medium and ELISA for TGFα.** SHIN-3 cells were grown to subconfluence in RPMI 1640 medium with 10% FBS in 75-cm$^2$ tissue culture flasks (Corning Glass Works, Corning, NY), washed 4 times with RPMI 1640, and incubated in 10 ml of serum-free (0.3% BSA) RPMI 1640 medium for 24 in a $CO_2$ incubator. The resulting conditioned medium was harvested, centrifuged at 400 × *g* for 10 min, acidified with acetate acid at a final concentration of 3%, and centrifuged to remove insoluble materials. Five ml of acidified supernatants were diluted with 0.1% trifluoroacetic acid and applied to Sep-Pak $C_{18}$ cartridges (Millipore Co., Milford, MA). The cartridges were washed and TGFα was eluted with 40% acetonitrile containing 0.1% trifluoroacetic acid. The eluates were lyophilized and dissolved in 1 ml of 10 mM PBS, pH 7.2. TGFα was measured by the sandwich-type ELISA as described elsewhere (16). Cells were removed by trypsinization and the cell numbers were counted on a hemocytometer.

**Growth Promotion and Inhibition Studies.** SHIN-3 cells were seeded in 6-well plates (Falcon) at a cell density of $10^5$/well in RPMI 1640 with 10% FBS and were plated. Twenty-four h later cells were washed 4–5 times with RPMI 1640 medium and the following studies were started. The effects of FBS contained in the culture medium on the cell growth were examined by incubating cells in RPMI 1640 containing 10% FBS, 2% FBS, or 0.3% BSA. Growth promotion studies were performed by incubating cells in the serum-free culture medium (RPMI 1640/0.3% BSA) containing 0.1, 1.0, or 10 nM TGFα. Growth inhibition studies were done by incubating SHIN-3 cells in the serum-free culture medium containing various amounts of mAbs against TGFα, EGFR, and EGF. Proportional amounts of nonimmune mouse IgG (Zymed, San Francisco, CA) were added in control cultures. Culture media with or without test materials were changed every 2 days. Cells were incubated at 37°C for the times indicated in the Figures in a humidified 95% air and 5% $CO_2$ atmosphere. Cells were isolated by trypsinization and the number of cells was counted on a hemocytometer.

**Statistical Analysis.** Data were shown as the mean ± SD. Homoscedasticity of data was analyzed by the Bartlett test. The significance of differences was assessed by analysis of variance, followed by multiple comparisons of Dunett and $P < 0.05$ was considered to be significant.

## RESULTS

**Northern Blot Analysis.** Gene transcripts from SHIN-3 cells were analyzed by Northern blotting. Poly(A)$^+$ RNA was selected after isolating total RNA from the cell and Northern analysis was performed by using $^{32}$P-labeled pre-TGFα, prepro-EGF cDNAs, and pE7 as probes. As shown in Fig. 1, pre-TGFα mRNA was observed at 4.8 kilobases (*Lane A*). Although prepro-EGF mRNA was not expressed in the cell (*Lane B*), 3 gene transcripts for EGFR were observed at 10, 5.6, and 2.9 kilobases (*Lane C*). β-Actin mRNA was observed at 2.0 kilobases.

**Immunocytochemistry.** Immunocytochemical stainings using, anti-TGFα, anti-EGF, and anti-EGFR mAbs revealed that SHIN-3 cells expressed TGFα and EGFR proteins in the cytoplasm and on the plasma membrane, respectively (Fig. 2, *A* and *B*) but they did not express EGF protein (data not shown). CA-125, which is a widely used ovarian tumor marker, also was detected (Fig. 2*C*) in accordance with the data that

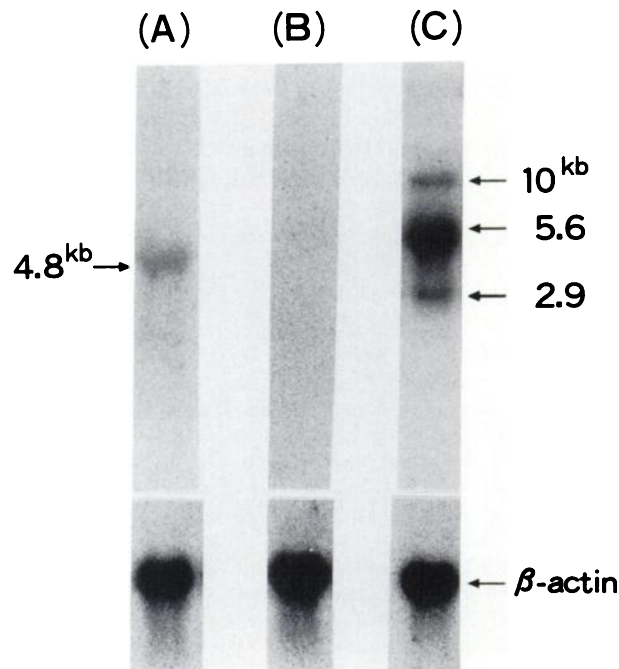


Fig. 1. Expression of pre-TGFα, prepro-EGF, and EGFR gene transcripts in SHIN-3 cells. Two μg of poly(A)$^+$ RNA was fractionated in 1% agarose/0.66 M formaldehyde gel, transferred to a nitrocellulose filter, and hybridized with $^{32}$P-labeled pre-TGFα (*A*), prepro-EGF (*B*), and EGFR (*C*) cDNA probes. Filters were washed at a highly stringent condition, autoradiographed, and rehybridized with β-actin. *kb*, kilobase.

SHIN-3 cells were derived from a CA-125-producing ovarian cancer (14).

**$^{125}$I-EGF Binding Studies and ELISA for TGFα.** We analyzed the EGFR on the cells by binding studies using $^{125}$I-labeled human EGF as a ligand. A class of high affinity receptor was found by Scatchard analysis (data not shown). $K_d$ was $2.9 \times 10^{-10}$ and $B_{max}$ was $7.7 \times 10^4$ sites/cell.

The amounts of immunoreactive TGFα in the conditioned media of SHIN-3 cells were measured by ELISA. As much as 1.12 ± 0.14 ng (SD; $n = 3$)/$10^7$ cells/24 h of TGFα immunoreactivities were present in the conditioned media.

All the above data suggested the presence of a TGFα/EGFR autocrine mechanism in this cell line. Next, we examined the biological significance of this mechanism on the growth of SHIN-3 cells, using monolayer cell cultures.

**Growth Promotion and Inhibition Studies.** Although SHIN-3 cells grew significantly ($P < 0.005$) less efficiently in a serum-free (0.3% BSA) condition than in media with 2% and 10% FBS, cells showed an aggressive growth even in serum-free medium as shown in Fig. 3. We, therefore, performed the following studies using serum-free monolayer cell cultures. Various concentrations of TGFα were added to the culture medium and no significant growth stimulations were observed by 0.1, 1.0, and 10 nM concentrations of TGFα (data not shown).

More important experiments for the critical assessment of the autocrine mechanism are the growth control studies by extracellular antagonists to a TGFα/EGFR autocrine mechanism (21). We, therefore, studied the effects of mAbs against TGFα and EGFR on the growth of SHIN-3 cells. As low as 1 μg/ml of anti-TGFα mAb significantly ($P < 0.005$) reduced cell growth and this growth inhibitory effect by anti-TGFα mAb was dose dependent. As much as 60% of cell growth was suppressed by 5 μg/ml of anti-TGFα mAb (Fig. 4*A*) and no



Downloaded from http://aacrjournals.org/cancerres/article-pdf/51/21/5951/2445250/cr0510215951.pdf by guest on 25 March 2023

more inhibition was observed by 10 μg/ml of Ab (data not shown). Growth inhibitions by TGFα mAb seemed specific: although 1 nM TGFα failed to restore the growth inhibitory effect by 1 μg/ml of anti-TGFα mAb, 10 nM TGFα completely restored it (Fig. 4*B*). Anti-EGFR mAb also exhibited the growth inhibitory effects in a dose-dependent manner at 1 and 5 μg/ml (Fig. 5). Growth inhibitory effect by EGFR mAb seemed specific because it was observed only by the anti-EGFR mAb (antibody 1) which inhibits the ligand binding (15) and not by anti-EGFR mAb (antibody 2) which does not inhibit the ligand binding (15) (data not shown). Anti-EGF mAb, which would not be an antagonist to this autocrine mechanism, did not show

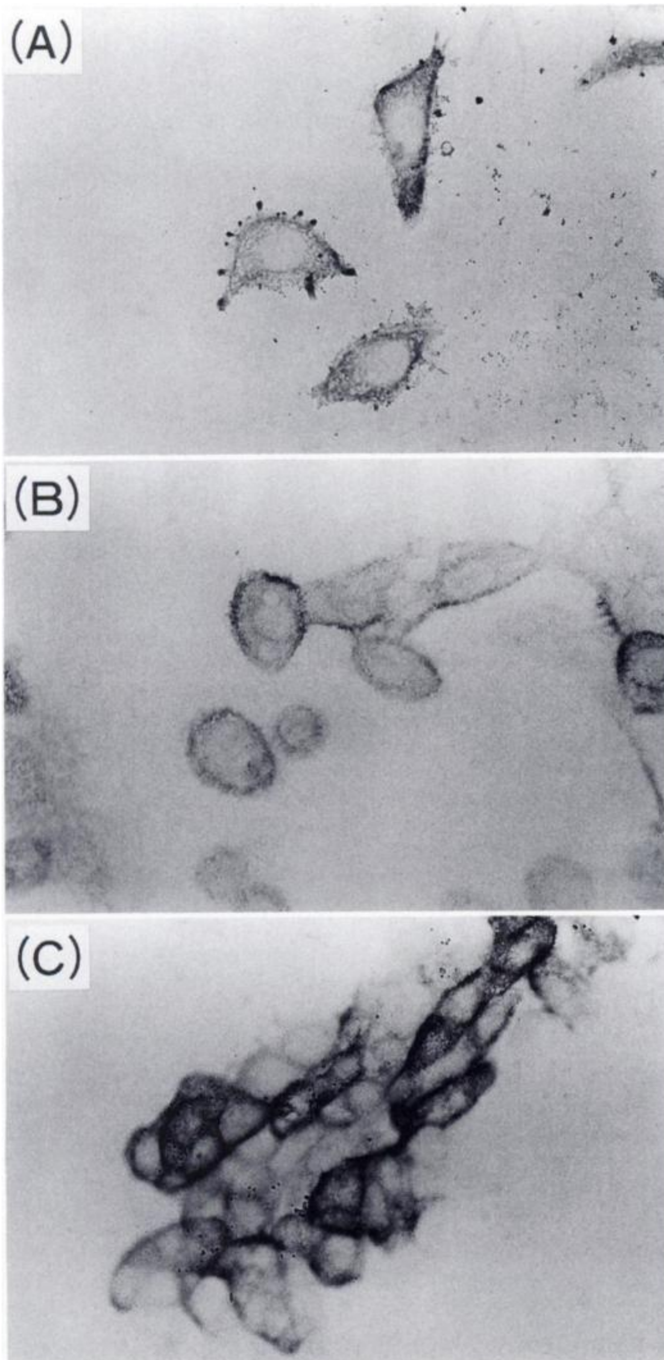


Fig. 2. Expression of TGFα, EGFR and CA-125 tumor marker proteins on the cell. Cells were attached to 8-chamber slides and immunocytochemical stainings were performed by using mAbs against TGFα (*A*), EGFR (*B*), and CA-125 (*C*).

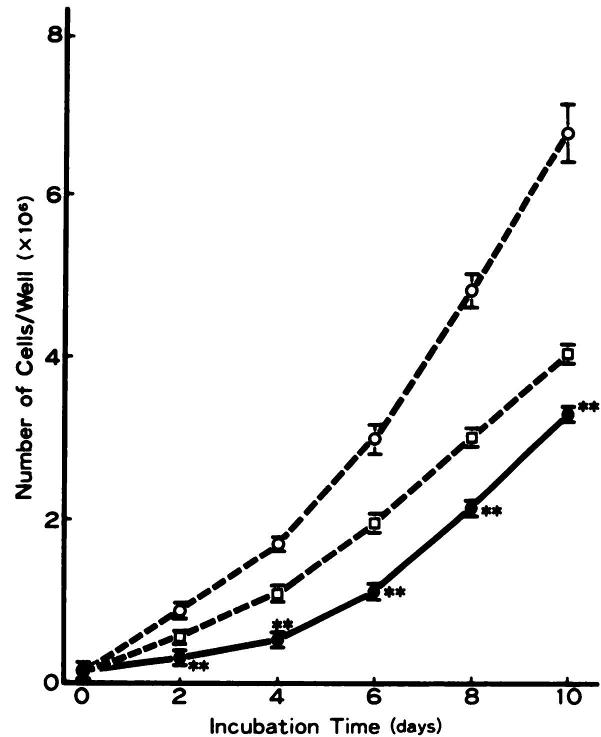


Fig. 3. Effects of the elimination of FBS in the culture medium on the growth of cells. SHIN-3 cells maintained in RPMI 1640/10% FBS were extensively washed with RPMI 1640 and $10^5$ cells were seeded in the 6-well plates. Then, cells were cultured in RPMI 1640 culture medium supplemented with 10% FBS (○), 2% FBS (□), or with 0.3% BSA (serum-free) (●) for the times indicated, and the number of cells was counted. **, $P < 0.005$ *versus* cultures with 2% and 10% FBS.

any growth inhibitory effect even at the concentration of 5 μg/ml (Fig. 6). These studies were performed in 3 wells in each group and the data were shown as mean ± SD. Each experiment was repeated 3–5 times, and a representative result was shown.

## DISCUSSION

For the critical assessment of an autocrine growth mechanism, it is most important to show the growth control effects of the antagonists of a presumed autocrine mechanism, as described by Sporn and Roberts (21). In this sense, although the TGFα/EGFR autocrine mechanism is most commonly expressed in various cancers (1–4, 9, 10), its biological significances on the growth of cancers have still been circumstantial. In this study, we critically assessed the biological significance of a TGFα/EGFR autocrine mechanism on the growth of a human ovarian serous cystadenocarcinoma derived cell line *in vitro*: mAbs against TGFα and EGFR (antibody 1) which were the antagonists to the TGFα/EGFR autocrine mechanism showed dose-dependent and specific growth inhibitory effects. Cell growth was reduced as large as 60% by 5 μg/ml of anti-TGFα mAb (Fig. 4*A*). This growth inhibitory effect was almost as potent as that by 4.0 μg/ml of *cis*-diamminedichloroplatinum *in vitro*[3] which is a potent carcinostatic agent, especially in urogenital cancers (22).

We investigated the biological significance of this TGFα/EGFR autocrine mechanism also *in vivo* by implanting SHIN-3 cells to *nu/nu* mice. Anti-TGFα mAb given to *nu/nu* mice drastically suppressed the tumor growth and, therefore, it was clear that this autocrine mechanism was biologically significant

[3] Unpublished data.



Downloaded from http://aacrjournals.org/cancerres/article-pdf/51/21/5951/2445250/cr0510215951.pdf by guest on 25 March 2023

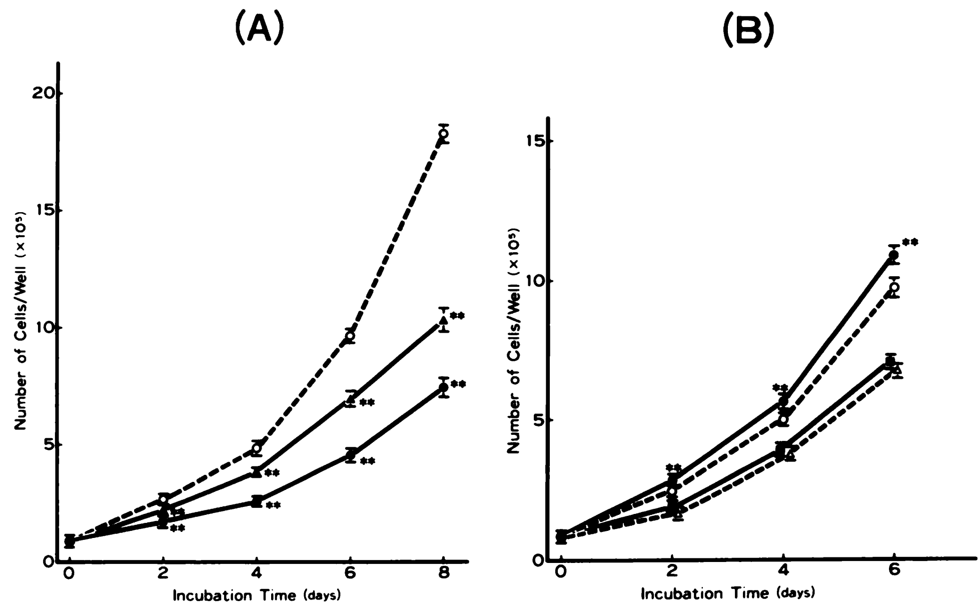


Fig. 4. *A*, growth inhibitory effects of anti-TGFα mAb on the growth of SHIN-3 cells. Cells were seeded in 6-well plates at a cell density of $10^5$/well in RPMI 1640 medium with 10% FBS and were allowed to plate. After a 24-h incubation, cells were extensively washed with RPMI 1640 and media were changed to serum-free RPMI 1640 containing 1 μg/ml (▲), 5 μg/ml (●) of anti-TGFα mAb or 5 μg/ml of nonimmune mouse IgG (control) (○). Cells were incubated for the times indicated and the number of cells was counted. Nonimmune IgG showed no effects on cell growth. **, $P < 0.005$ *versus* control cultures. *B*, restorative effects of TGFα on the growth inhibition by 1 μg/ml of anti-TGFα mAb. Cells were incubated in serum-free RPMI 1640 medium containing 1 μg/ml of anti-TGFα mAb with 1 nM (■), 10 nM (●) TGFα, or without TGFα (△). One μg/ml of nonimmune mouse IgG was added to control cultures (○). Cell numbers were counted at the times indicated. **, $P < 0.005$ *versus* the culture with 1 μg/ml of anti-TGFα mAb only (△).

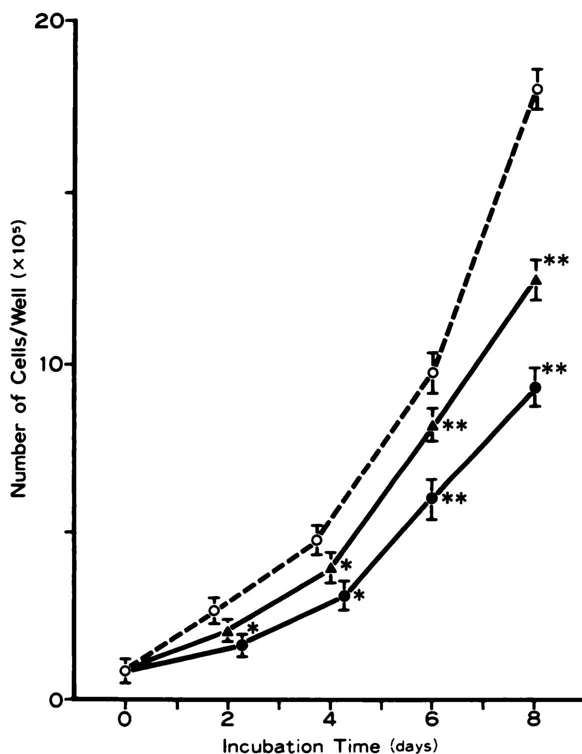


Fig. 5. Growth inhibition by anti-EGFR mAb. Cells were incubated in serum-free RPMI 1640 medium containing 1 μg/ml (▲), 5 μg/ml (●) of anti-EGFR mAb, or 5 μg/ml of nonimmune mouse IgG (control) (○) for the times indicated, and the cell numbers were counted. *, $P < 0.05$; **, $P < 0.005$ *versus* control cultures.

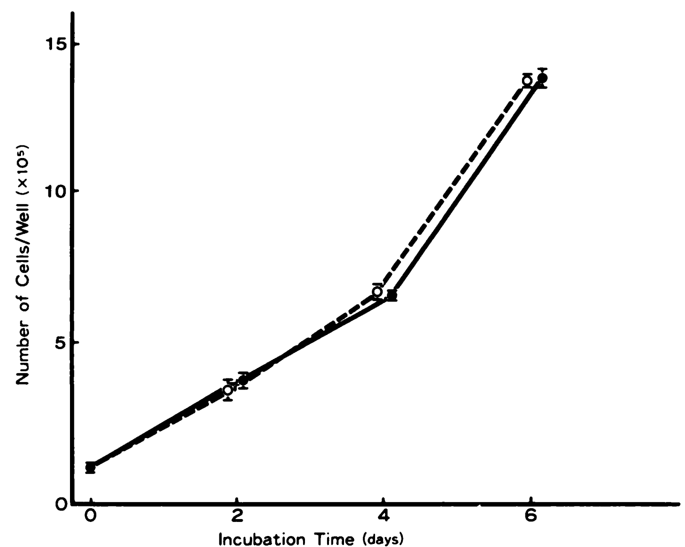


Fig. 6. Effects of anti-EGF mAb on the growth of cells. Cells were incubated in serum-free RPMI 1640 medium containing 5 μg/ml of anti-EGF mAb (●) or 5 μg/ml of nonimmune mouse IgG (○) for the times indicated, and the cell numbers were counted.

on the growth of SHIN-3 cells also *in vivo* (23). However, the fact that this cell line grew less efficiently in lower FBS concentrations (Fig. 3) suggested that SHIN-3 cells possessed other growth mechanism(s) than the TGFα/EGFR autocrine mechanism.

The fact that anti-EGF mAb did not show growth inhibitory effects suggested that this cell line did not express biologically significant amounts of EGF, and would support the results in the Northern blot analysis and immunocytochemical staining that SHIN-3 cells did not express prepro-EGF mRNA nor EGF protein.

We also studied the autocrine mechanism in some primary human ovarian cancer tissues obtained at the time of operation and found the presence not of an EGF/EGFR autocrine mechanism but of a TGFα/EGFR mechanism, as far as we examined (24). Moreover, we have found that the TGFα/EGFR autocrine mechanism played an important role on the growth also in primary human ovarian cancers *in vitro* (24). This TGFα/EGFR autocrine growth mechanism was present rather generally in primary human ovarian cancers (24). These results in primary cancers suggested that the expression and biological importance of the TGFα/EGFR autocrine mechanism were not limited to this cell line. We, therefore, might suggest a possibility that antagonists to TGFα/EGFR autocrine mechanism could be effective in treating human ovarian cancers which express a TGFα/EGFR autocrine mechanism.



Downloaded from http://aacrjournals.org/cancerres/article-pdf/51/21/5951/2445250/cr0510215951.pdf by guest on 25 March 2023

## ACKNOWLEDGMENTS

We gratefully acknowledge Dr. Emiko Semba, Department of Anatomy, Osaka University Medical School, for her generous support in immunocytochemical studies.

## REFERENCES

1. Derynck, R., Goeddel, D. V., Ullrich, J. U., Gutterman, J. V., Williams, R. D., Bringman, T. S., and Berger, W. H. Synthesis of messenger RNAs for transforming growth factors α and β and the epidermal growth factor receptor by human tumors. Cancer Res., *47:* 707–712, 1987.
2. Mydlo, J. H., Michaeli, J., Cardo, C. C., Goldenberg, A. S., Heston, W. D. W., and Fair, W. R. Expression of transforming growth factor α and epidermal growth factor receptor RNA in neoplastic and non neoplastic human kidney tissue. Cancer Res., *49:* 3407–3411, 1989.
3. Gomella, L. G., Sargent, E. R., Wade, T. P., Anglard, P., Linehan, W. M., and Kasid, A. Expression of transforming growth factor α in normal human adult kidney and enhanced expression of transforming growth factor α and $\beta_1$ in renal cell carcinoma. Cancer Res., *49:* 6972–6975, 1989.
4. Tateishi, M., Ishida, T., Mitsudomi, T., Kaneko, S., and Sugimachi, K. Immunohistochemical evidence of autocrine growth factors in adenocarcinoma of the human lung. Cancer Res., *50:* 7077–7080, 1990.
5. Di Marco, E., Pierce, J. H., Fleming, T. P., Kraus, M. H., Molloy, C. J., Aaronson, S. A., and Di Fiore, P. P. Autocrine interaction between TGFα and the EGF-receptor: quantitative requirements for induction of the malignant phenotype. Oncogene, *4:* 831–838, 1989.
6. Ohmura, E., Okada, M., Onoda, N., Kamiya, Y., Murakami, H., Tsushima, T., and Shizume, K. Insulin-like growth factor I and transforming growth factor α as autocrine growth factors in human pancreatic cancer cell growth. Cancer Res., *50:* 103–107, 1990.
7. Moody, T. W., Carney, D. N., Cuttitta, F., Quattrocchi, K., and Minna, J. D. High affinity receptors for bombesin/GRP-like peptides on human small cell lung cancer. Life Sci., *37:* 105–113, 1985.
8. Cuttitta, F., Carney, D. N., Mulshine, J., Moody, T. W., Fedorko, J., Fischler, A., and Minna, J. D. Bombesin-like peptides can function as autocrine growth factors in human small-cell lung cancer. Nature (Lond.), *316:* 823–826, 1985.
9. Massague, J. Epidermal growth factor-like transforming growth factor II. Interaction with epidermal growth factor receptors in human placenta membranes and A431 cells. J. Biol. Chem., *258:* 13614–13620, 1983.
10. Yoshida, K., Kyo, E., Tsuda, T., Tsujino, T., Ito, M., Niimoto, M., and Tahara, E. EGF and TGFα, the ligands of hyperproduced EGFR in human esophageal carcinoma cells, act as autocrine growth factors. Int. J. Cancer, *45:* 131–135, 1990.
11. Sherwin, S. A., Twardzik, D. R., Bohn, W. H., Cockley, K. D., and Todaro, G. J. High-molecular-weight transforming growth factor activity in the urine of patients with disseminated cancer. Cancer Res., *43:* 403–407, 1983.
12. Arteaga, C. L., Hanauske, A. R., Clark, G. M., Osborne, C. K., Hazarika, P., Pardue, R. L., Tio, F., and Von Hoff, D. D. Immunoreactive α transforming growth factor activity in effusions from cancer patients as a marker of tumor burden and patient prognosis. Cancer Res., *48:* 5023–5028, 1988.
13. Bauknecht, T., Kiechle, M., Bauer, G., and Siebers, J. W. Characterization of growth factors in human ovarian carcinomas. Cancer Res., *46:* 2614–2618, 1986.
14. Kiyozuka, Y., Imai, S., Noda, T., Morimoto, J., Moriyama, I., Ichijo, M., and Tsubura, Y. Establishment and characterization of human ovarian serous cystadenocarcinoma cell line (SHIN-3) which produces CA 125 and TPA *in vitro*. J. Jpn. Soc. Clin. Cytol., *27:* 926–935, 1988.
15. Kawamoto, T., Sato, J. D., Le, A., Polikoff, J., Sato, G. H., and Mendelsohn, J. Growth stimulation of A431 cells by epidermal growth factor: identification of high-affinity receptors for epidermal growth factor by an anti-receptor monoclonal antibody. Proc. Natl. Acad. Sci. USA, *80:* 1337–1341, 1983.
16. Katoh, M., Inagaki, H., Kurosawa-Ohsawa, K., Katsuura, M., and Tanaka, S. Detection of transforming growth factor alpha in human urine and plasma. Biochem. Biophys. Res. Commun., *167:* 1065–1072, 1990.
17. Extraction, purification, and analysis of messenger RNA from eukaryotic cells. *In:* T. Maniatis, E. F. Fritsch, and J. Sambrook (eds.), Molecular Cloning: A Laboratory Manual, Ed. 2, pp. 7.26–7.29. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1989.
18. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., and Rutter, W. J. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry, *18:* 5294–5299, 1979.
19. Formaldehyde gel for electrophoretic separation of RNA and Northern blot. *In:* L. G. Davis, M. D. Dibner, and J. F. Battey (eds.), Basic Methods in Molecular Biology, pp. 143–146, New York: Elsevier Science Publishing Co., 1986.
20. Wahl, G. M., Stern, M., and Stork, G. R. Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl-paper and rapid hybridization by using dextran sulfate. Proc. Natl. Acad. Sci. USA, *76:* 3683–3687, 1979.
21. Sporn, M. B., and Roberts, A. B. Autocrine growth factors and cancer. Nature (Lond.), *313:* 745–747, 1985.
22. Loehrer, P. J., and Einhorn, L. H. Diagnosis and treatment, Cisplatin. Ann. Intern. Med., *100:* 704–713, 1984.
23. Kurachi, H., Morishige, K., Amemiya, K., Adachi, H., Hirota, K., Miyake, A., and Tanizawa, O. Importance of transforming growth factor α/epidermal growth factor receptor autocrine growth mechanism in an ovarian cancer cell line *in vivo*. Cancer Res., *51:* 5956–5959, 1991.
24. Morishige, K., Kurachi, H., Amemiya, K., Fujita, Y., Yamamoto, T., Miyake, A., and Tanizawa, O. Evidence for the involvement of transforming growth factor α and epidermal growth factor receptor autocrine growth mechanism in primary human ovarian cancers *in vitro*. Cancer Res., *51:* 5322–5328, 1991.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/51/21/5951/2445250/cr0510215951.pdf by guest on 25 March 2023