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Effects of Sex Steroids and Growth Factors on Invasive Activity and 5′-Deoxy-5-fluorouridine Sensitivity in Ovarian Adenocarcinoma OMC-3 Cells

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Effects of sex steroids (estradiol-17β, E_2; progesterone, Prog) and growth factors (epidermal growth factor, EGF; transforming growth factor-α, TGF-α) on invasive activity and 5′-deoxy-5-fluorouridine (5′-dFUrd) sensitivity of ovarian adenocarcinoma OMC-3 cells were investigated. Tumor cell migration along a gradient of substratum-bound fibronectin and invasion into reconstituted basement membrane were inhibited by 10 *μM* Prog, but stimulated by 0.1–10 n*M* EGF and TGF-α in a concentration-dependent manner. E_2 did not have any effect on tumor cell migration or invasion. The zymography of tumor conditioned medium showed that the treatment of OMC-3 cells with EGF and TGF-α resulted in increases of type IV collagenase, stromelysin and urokinase-type plasminogen activator (uPA). EGF and TGF-α up-regulated thymidine phosphorylase (dThdPase) expression of tumor cells and consequently enhanced the antiproliferative action of 5′-dFUrd, which is converted to 5-fluorouracil by dThdPase. E_2 and Prog did not have significant effects on the expression of proteolytic enzymes and dThdPase, or on the 5′-dFUrd sensitivity of tumor cells. The inhibitory effect of Prog on tumor cell invasion may depend on its inhibitory action on the motility of tumor cells. These results suggest that EGF and TGF-α simultaneously up-regulate the potential of ovarian adenocarcinoma cells to invade extracellular matrices and their dThdPase expression, both of which are associated with the specific action of 5′-dFUrd selectively to kill tumor cells with high invasive and metastatic potential.

Key words: Ovarian adenocarcinoma — Invasion — Matrix metalloproteinase — Thymidine phosphorylase — 5′-Deoxy-5-fluorouridine

Tumor cell invasion into extracellular matrices (ECM) and basement membranes, a crucial step in the complex multistage process which leads to the formation of metastasis, involves a distinct sequence of events including cell attachment, migration and the degradation of ECM components by proteolytic enzymes secreted by tumor cells.[1–3] Matrix metalloproteinases (MMPs) are proteolytic enzymes which can degrade native collagens and other ECM components.[4,5] Since their substrates are the major components of ECM, the increased expression of MMPs by malignant tumor cells is believed to play an essential role in invasion and metastasis.[6,7] The urokinase-type plasminogen activator (uPA) also plays an important role in the invasion process.[3] The proteolytic cascade of ECM components is triggered by the uPA-mediated conversion of plasminogen to plasmin and the subsequent activation of procollagenases.[8,9] Regulation of proteinase expression is achieved through specific tissue inhibitors, activation of latent forms of proenzymes, and modulation of gene transcription by various factors.[7,10] Inflammatory cytokines, tumor necrosis factor (TNF) α, interleukin (IL)-1α and interferon (INF) γ, which are often detected in the tumor environment,[11,12] are reported to up-regulate the expression of type IV collagenase, a member of MMP gene family, and to enhance the metastatic potential of tumor cells in either *in vitro* or *in vivo* tumor models.[13–17] In addition, factors which down-regulate cytokine expression, such as indomethacin, were demonstrated to suppress metastasis.[18] Thus, the potential of tumor cells to invade the ECM seems to be regulated, at least in part, by tumor environmental factors.

5′-Deoxy-5-fluorouridine (5′-dFUrd) is an orally available cytostatic widely used for the treatment of breast, colorectal and gastric cancers.[19] 5′-dFUrd is biotransformed to 5-fluorouracil (5-FUra) by pyrimidine nucleoside phosphorylase (PyNPase), mainly thymidine phosphorylase (dThdPase) in human tumors.[20] Eda *et al.*[21,22] reported that inflammatory cytokines, such as TNFα, IL-1α and INFγ, up-regulate PyNPase expression in human and mouse tumor cells and increase the sensitivity of tumor cells to 5′-dFUrd. Recently, Ishikawa *et al.*[23] demonstrated that the expression of type IV collagenase and PyNPase were simultaneously up-regulated in tumor cells by cytokines, and that 5′-dFUrd showed high anti-metastatic activity by selectively killing tumor cells that

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were over-expressing the metastatic factor type IV collagenase, because of high PyNPase activity. However, in this field, there have been very few reports on the biological significance of sex steroids and growth factors, which play an important role in the control of proliferation and invasion in tumor cells of gynecological origin.[9, 24, 25] In the present study, we investigated the effects of sex steroids and growth factors on migration, invasion and proteinase expression of cultured ovarian adenocarcinoma cells, and examined whether these tumor environmental factors affect the dThdPase expression and 5′-dFUrd sensitivity of tumor cells.

MATERIALS AND METHODS

Cell culture OMC-3 originating from a human mucinous cystadenocarcinoma of the ovary was established at our laboratory.[26] OMC-3 cells were maintained as monolayer cultures in Ham's F-12 medium (Flow Laboratories Inc., Irvine, Scotland) supplemented with 10% fetal bovine serum (Mitsubishi Chemical Co., Tokyo) at 37°C in a humidified incubator with 5% CO_2 in air. Tumor conditioned medium (TCM) was prepared from the culture supernatant of the cells. Briefly, 1.0×10^6 cells were uniformly seeded into 3.5-cm plastic dishes (Iwaki Glass, Tokyo) and cultured for 48 h with growth medium. Confluent monolayers grown in the dishes were then rinsed twice with serum-free Ham's F-12 medium and incubated at 37°C for 48 h with 1 ml of serum-free medium. The TCM samples were stored at –80°C until use.

Chemical reagents Estradiol-17β (E_2) and progesterone (Prog) were purchased from Sigma Chemical Co., St. Louis, MO. Epidermal growth factor (EGF) and transforming growth factor (TGF)-α were purchased from Wakunaga Yakuhin Co., Ltd., Osaka. Purified human fibronectin, gelatin, casein and fibrinogen were purchased from Iwaki Glass. Basement Membrane Matrigel was obtained from Collaborative Research Inc., Bedford, MA. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from Wako Pure Chemical Industries, Ltd., Osaka. 5′-dFUrd and 5-FUra were kindly provided by Nippon Roche Co., Ltd., Osaka and Kyowa Hakko Co., Ltd., Tokyo, respectively.

Haptotactic migration assay Tumor cell migration along a gradient of substratum-bound fibronectin was assayed in Chemotaxicell culture chambers (Kurabo, Osaka) according to the methods reported by McCarthy and Furcht[27] with some modifications as previously described.[25] Polycarbonate filters with 8.0 μm pore size were precoated with 10 μg of fibronectin on the lower surface. Log-phase cell cultures of tumor cells were harvested and resuspended to a final concentration of 3.0×10^6/ml in serum-free Ham's F-12 with 0.1% bovine serum albumin (BSA) (Sigma). A 200 μl aliquot of cell suspension with or without various amounts of sex steroids or growth factors was added to the upper compartment, and 600 μl of serum-free Ham's F-12 with 0.1% BSA was added to the lower compartment. The chambers were then incubated for 48 h at 37°C in 5% CO_2 air. The filters were fixed with ethanol, then stained with hematoxylin, and the cells on the upper surface of the filters were removed by wiping with a cotton swab. The cells that had migrated to the lower surface were manually counted under a microscope at a magnification of 400.

Invasion assay The invasive activity of tumor cells was assayed according to the method reported by Albini *et al.*[28] with some modifications as previously described.[25] Briefly, the lower surface of the filters was precoated with fibronectin as described above. The Matrigel diluted to 500 μg/ml with cold phosphate-buffered saline (PBS) was applied to the upper surface of the filters (5 μg/filter). The subsequent procedures were the same as those of the haptotactic migration assay.

Zymograms The proteolytic activity of TCM was examined by electrophoresis in a gelatin, casein or fibrinogen-containing polyacrylamide gel, based on the methods described by Heussen and Dowdle.[29] The TCM samples were mixed with sodium dodecyl sulfate (SDS) sample buffer containing 1 m*M* phenylmethylsulfonyl fluoride and applied, without heating or reduction, to polyacrylamide gels containing 1 mg/ml of gelatin, casein or fibrinogen. After electrophoresis, the gels were washed twice with 2.5% Triton X-100 for 60 min to remove the SDS, incubated in the incubation buffer containing 0.15 *M* NaCl, 50 m*M* Tris-HCl, 10 m*M* $CaCl_2$, 0.05% NaN_3 for 24 h, and then stained in 0.1% Coomassie Blue.

dThdPase assay dThdPase expression of tumor cells was assayed according to the method reported by Nishida *et al.*[30] Confluent monolayers of tumor cells grown in 3.5-cm plastic dishes were rinsed twice with serum-free Ham's F-12 with 0.1% BSA and incubated at 37°C for 48 h with 1 ml of the same medium with or without various amounts of sex steroids or growth factors. The cells were then harvested, washed with PBS, and collected by centrifugation. The pellet was homogenized with 4 volumes of a buffer containing 1 m*M* EDTA, 5 m*M* 2-mercaptoethanol, 100 μ*M* *p*-amidinophenylmethanesulfonyl fluoride (APMSF) and 50 m*M* potassium phosphate (pH 7.5). The homogenate was centrifuged at 100,000*g* for 1 h, at 4°C, and the protein fraction precipitating between 20% and 45% saturation with ammonium sulfate at 4°C was collected by centrifugation. The precipitate was dissolved in buffer containing 1 m*M* EDTA, 5 m*M* 2-mercaptoethanol, 10 μ*M* APMSF and 20 m*M* potassium phosphate (pH 7.5), dialyzed against the same buffer, and used as a source of crude dThdPase. The protein concentration was determined by the method of Lowry *et al.*[31] Each sample was then dispensed onto the microplate well previously

coated with anti-dThdPase monoclonal antibody. The plate was incubated at 37°C for 1 h, washed with 0.05% Tween 20 in PBS, and then incubated with anti-dThdPase antibody at 1 μg/ml for 1 h at 37°C. It was further washed, incubated with 2,000-fold diluted anti-mouse IgG conjugated with horseradish peroxidase (HRP) for 30 min at 37°C, washed again, and incubated with a substrate solution containing 3,3′,5,5′-tetramethylbenzidine and hydrogen peroxide for 10 to 20 min at room temperature. The reaction was stopped by the addition of 1 *M* phosphate solution, and the absorbance at 450 nm was measured by using a microplate reader. The amount of dThdPase was determined by comparison with values obtained for standard dThdPase solutions.

Cytotoxic assay Effects of sex steroids and growth factors on the antiproliferative action of 5′-dFUrd and 5-FUra on tumor cells were examined by MTT assay with some modifications as previously described.[26] Cells (5.0×10^3) in a volume of 100 μl of growth medium per well were uniformly seeded into 96-well microplates (Nunc), and incubated at 37°C for 24 h. Each well was washed twice with serum-free Ham's F-12 containing 0.1% BSA and cultured for another 24 h in the same medium. The medium was then removed and replaced with 100 μl of serum-free Ham's F-12 containing 0.1% BSA and various amounts of cytostatics. The cells were cultured at 37°C for 7 days in the presence or absence of sex steroids or growth factors. The medium was changed every other day. At the end of incubation period, 50 μl of MTT dissolved in PBS at a concentration of 2 mg/ml was added to each well, and the plates were incubated at 37°C for 6 h. The medium was then removed, 200 μl of dimethyl sulfoxide (Sigma) was added to each well, and the plates were agitated for 5 min. The absorbance was read at 570 nm in a microplate reader. The IC_{50} values for the cytostatics were obtained as the concentration at which cell growth was inhibited by 50% as compared with the control value.

Receptor assay Estrogen receptor (ER) and progesterone receptor (PR) of OMC-3 cells were assayed by the dextran-coated charcoal (DCC) method.[32] Cells (2.0×10^7) were homogenized, and aliquots of the cytosol and the nucleus fraction were incubated with increasing concentrations of ^{3}H-estradiol or ^{3}H-R5020 (Amersham, Aylesbury, UK). After incubation, receptor-bound steroids were separated by absorption of unbound steroids onto DCC. Bound radioactivity was measured in a liquid scintillation counter (TRI-CARB 300c, Packard, CT). EGF receptor (EGFR) of OMC-3 cells was assayed as previously described.[25] Briefly, 2.0×10^7 cells were homogenized, and aliquots of the membrane fraction were incubated with increasing concentrations of ^{125}I-EGF (New England Nuclear Inc., Boston, MA) with or without unlabeled EGF (100 n*M*) for 16 h at room temperature. Radioactivity of the pellets obtained by centrifugation at 2,000*g* for 20 min at 0°C was measured in a gamma scintillation counter (ANSR, Dainabot Ltd., Tokyo), and Scatchard analysis was carried out.

Statistical analysis All experiments were performed in triplicate. The significance of differences between groups was calculated by applying a non-parametric test.

RESULTS

Effects of sex steroids and growth factors on tumor cell migration Since the invasive activity of tumor cells in an *in vitro* culture system is considered to reflect the combined effects of tumor cell motility and enzymatic degradation of ECM components, we first examined the effects of sex steroids and growth factors on tumor cell migration. As shown in Fig. 1, haptotactic migration of OMC-3 cells along a gradient of substratum-bound fibronectin was inhibited by 10 μM Prog, but stimulated by 0.1–10 n*M* EGF and TGF-α in a concentration-dependent manner. E_2 did not have any significant effect on tumor cell migration.

Effects of sex steroids and growth factors on tumor cell invasion As shown in Fig. 2, the invasive activity of tumor cells into reconstituted basement membrane components (Matrigel) was inhibited by 10 μM Prog, but stimulated by the presence of EGF and TGF-α in a concentration-dependent manner. E_2 did not have any effect on tumor cell invasion.

Effects of sex steroids and growth factors on the degradation of gelatin, casein or fibrinogen in zymograms We next examined the enzymatic degradation of gelatin, casein or fibrinogen substrates in zymograms, using TCM obtained from a 48-h incubation of tumor cells with or without sex steroids or growth factors. As can be seen in Figs. 3–5, the zymography showed that the treatment of tumor cells with EGF and TGF-α resulted in concentration-dependent increases of 72 kDa type IV collagenase, stromelysin and uPA. E_2 and Prog did not have any significant effect on these enzymes.

Effects of sex steroids and growth factors on dThdPase expression Since it can be assumed that the sensitivity of tumor cells to 5′-dFUrd depends on the PyNPase activity of the cells, we measured the enzyme expression of OMC-3 cells treated for 48 h with sex steroids or growth factors. As shown in Table I, the dThdPase expression of tumor cells was increased up to 1.73-fold by 0.1–10 n*M* EGF and TGF-α in a concentration-dependent manner, whereas E_2 and Prog did not have any significant effect on the dThdPase expression of the cells.

Effects of sex steroids and growth factors on the antiproliferative action of 5′-dFUrd and 5-FUra Next, we examined whether 5′-dFUrd selectively kills OMC-3 cells exposed to sex steroids and growth factors. Table II shows

the IC_{50} values for cytostatics in OMC-3 cells. EGF and TGF-α enhanced the antiproliferative activity of 5′-dFUrd by up to 6.51-fold, while the sensitivity of tumor cells to 5-FUra was increased only slightly (up to 1.49-fold) in response to growth factors. E_2 and Prog did not have any effect on the 5′-dFUrd and 5-FUra sensitivity of the cells.

ER, PR and EGFR of OMC-3 cells OMC-3 cells did not contain ER or PR in either cytosol or nuclei. A Scatchard plot of EGF binding to OMC-3 cells indicated a single class of binding sites with a dissociation constant of 4.5 n*M*, and the total binding sites amounted to 220 fmols per mg protein.

DISCUSSION

Sex steroids and growth factors play an important role in the proliferation and differentiation of epithelial cells of the female genital tract. Recent investigations[9, 24, 25, 33] have been focused on autocrine growth factors and malignant characteristics of tumor cells of gynecological origin, which are closely related to the biological functions of ovarian sex steroids. Bulletti *et al.*[24] and Jasonni *et al.*[33] demonstrated that E_2, Prog and synthetic progestins might be involved not only in growth control, but also in basement membrane degradation or formation of endometrial adenocarcinoma tissues induced by EGF and TGF-α as

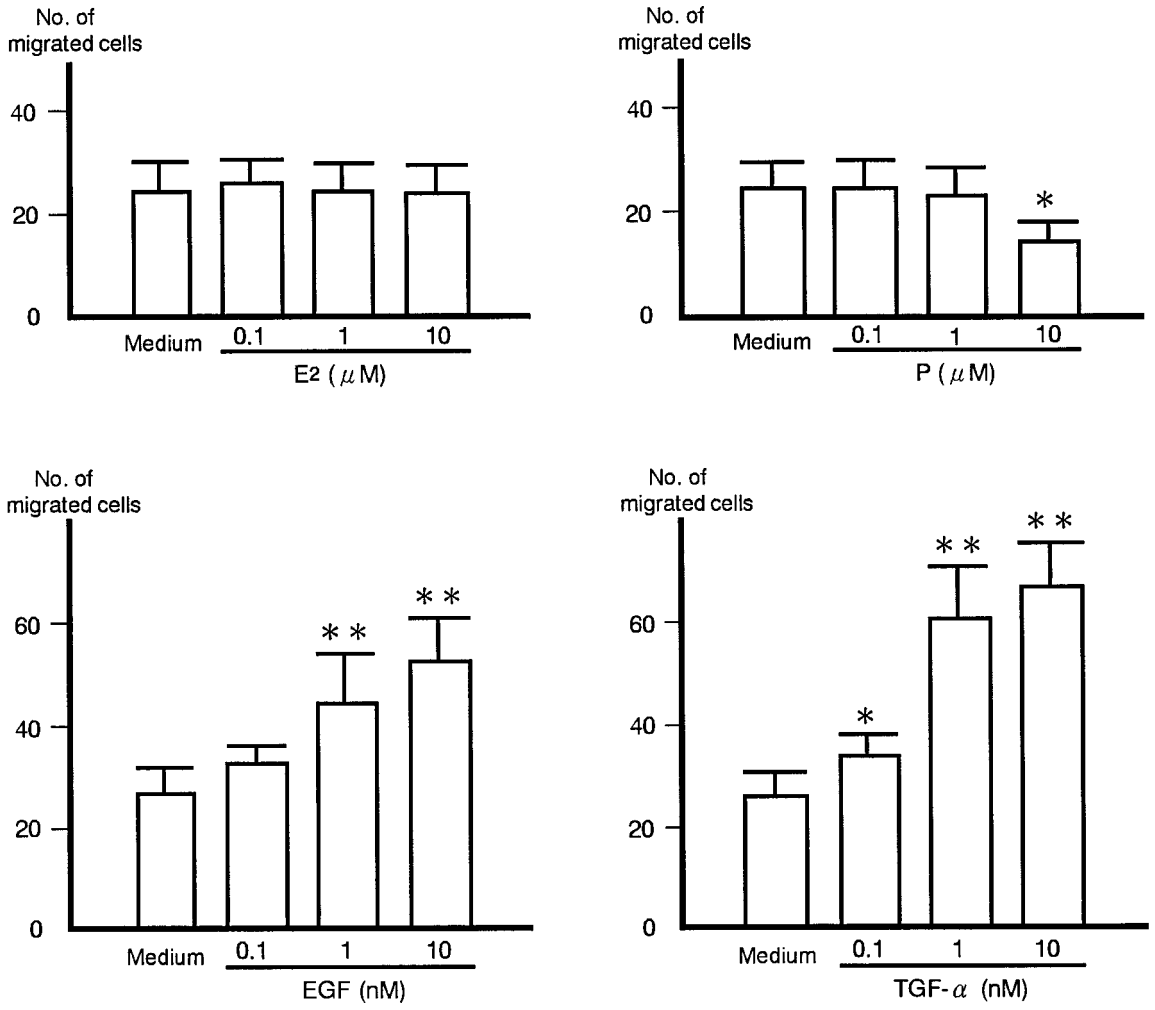

Fig. 1. Effects of sex steroids and growth factors on the haptotactic migration of OMC-3 cells. Cells (6.0×10^5) in Ham's F-12 with 0.1% BSA were seeded with or without 0.1–10 μ*M* E_2 and Prog or 0.1–10 n*M* EGF and TGF-α into the upper compartment of Chemotaxicell culture chambers. Filters in chambers were precoated with 10 μg of fibronectin on the lower surface. The migrated cells on the lower surface were counted under a microscope after a 48-h incubation. Bars: SE. * $P<0.05$ versus control, ** $P<0.01$ versus control.

second mediators of sex steroids. Moreover, Yoshida *et al.*[34] reported that EGF and TGF-α, which are secreted by tumor cells and/or surrounding interstitial cells, increased the expression of mRNA for several MMPs in human gastric carcinoma cell line, and Yudoh *et al.*[35] demonstrated that EGF stimulated the invasiveness of RCT sarcoma cells through acceleration of the degradative cascade of ECM. We also reported that synthetic progestins and growth factors affected the invasive activity of endometrial adenocarcinoma cells.[9, 25] These previous reports indicate that sex steroids and growth factors are strongly associated with the enzymatic degradation of ECM components and the invasion process of tumor cells. However, *in vitro* effects of these tumor environmental factors on the invasive activity of ovarian adenocarcinoma cells have not been elucidated.

The motility (haptotactic migration) and the invasive activity of OMC-3 cells were inhibited by 10 μM Prog, but stimulated by 0.1–10 n*M* EGF and TGF-α in a concentration-dependent manner. E_2 did not have any effect on tumor cell migration or invasion under our experimental conditions. The zymography of TCM showed that EGF

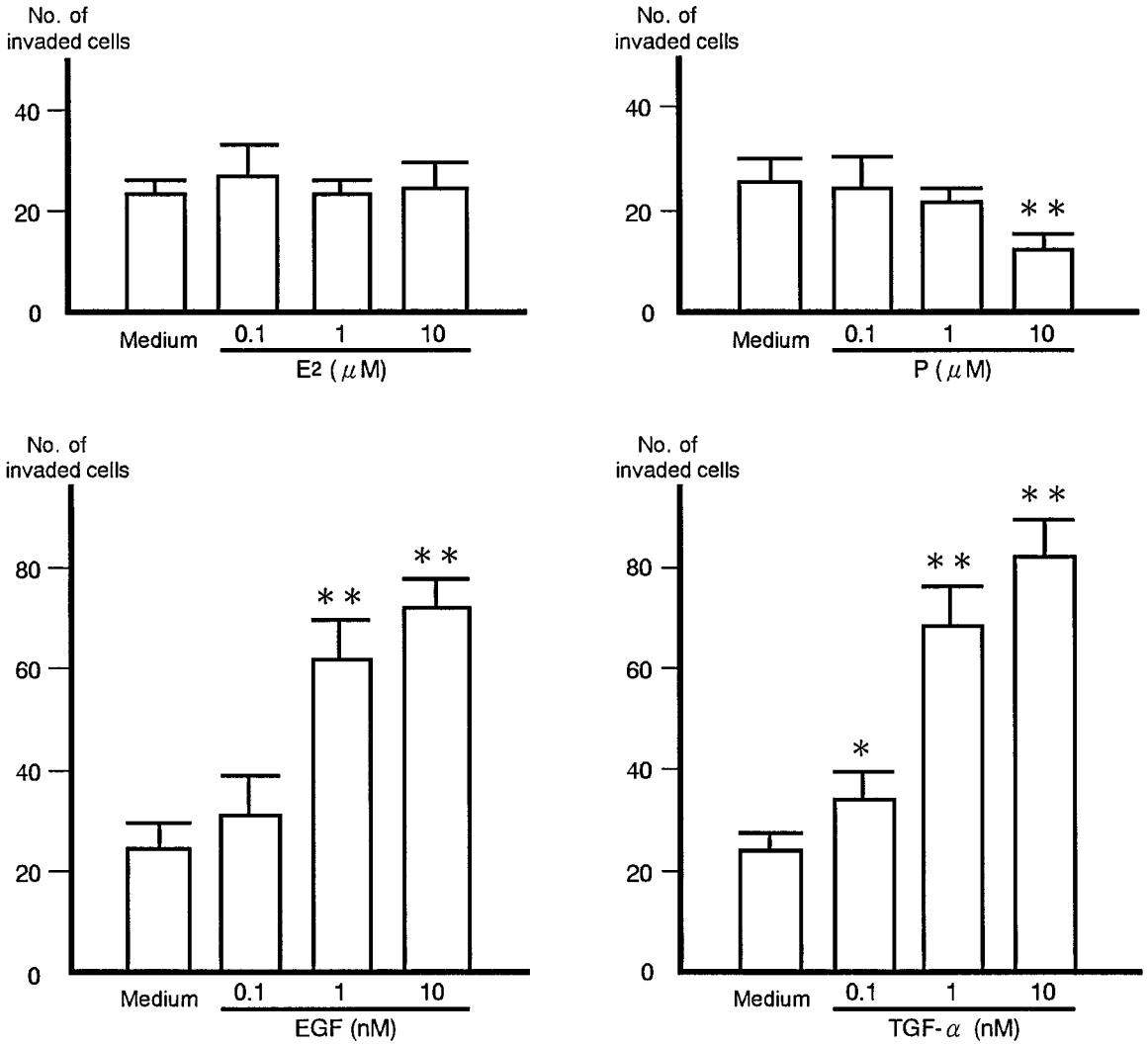

Fig. 2. Effects of sex steroids and growth factors on the invasive activity of OMC-3 cells into Matrigel/fibronectin-coated filters. Cells (6.0×10^5) in Ham's F-12 with 0.1% BSA were seeded with or without 0.1–10 μM E_2 and Prog or 0.1–10 n*M* EGF and TGF-α into the upper compartment of the Chemotaxicell culture chambers. Filters in the chambers were precoated with 10 μg of fibronectin on the lower surface, and with 5 μg of Matrigel on the upper surface. The invaded cells on the lower surface were counted under a microscope after a 48-h incubation. Bars: SE. * $P<0.05$ versus control, ** $P<0.01$ versus control.

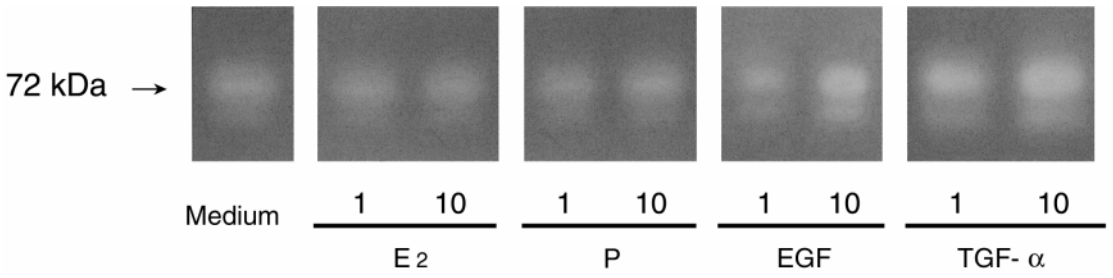

Fig. 3. The gelatinolytic activity of TCM from OMC-3 cells. Confluent monolayers of tumor cells were cultured for 48 h with or without 1–10 μM E_2 and Prog or 1–10 nM EGF and TGF-α in serum-free Ham's F-12. TCM samples were mixed with SDS-sample buffer, and then immediately applied, without heating or reduction, to gelatin-containing SDS-polyacrylamide gels. After electrophoresis, the gels were incubated and stained with Coomassie Brilliant Blue, and active enzymes were detected as unstained bands.

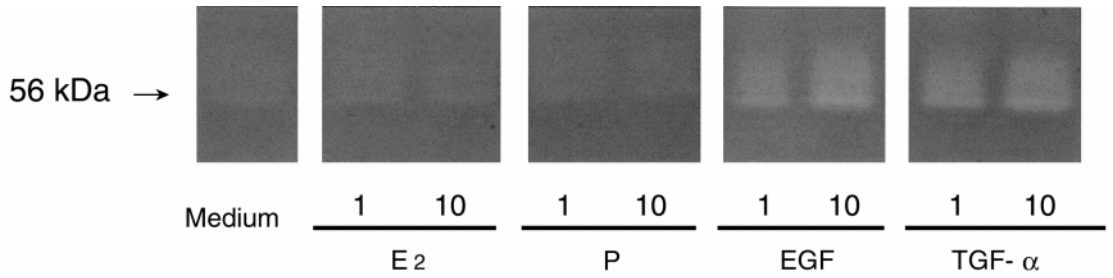

Fig. 4. The caseinolytic activity of TCM from OMC-3 cells. TCM samples were obtained as described in Fig. 3, and applied to casein-containing SDS-polyacrylamide gels. Active enzymes were detected as unstained bands as described in Fig. 3.

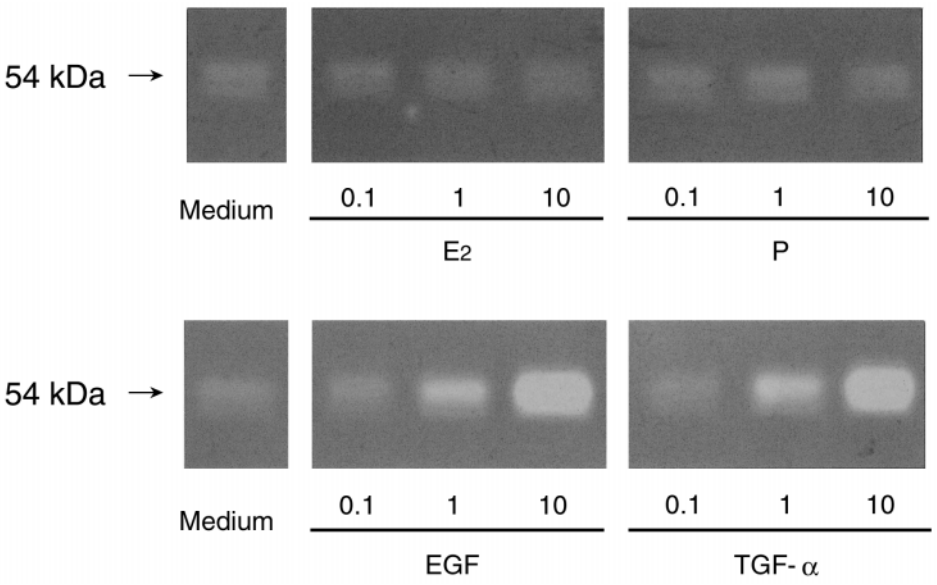

Fig. 5. The plasmin activity of TCM from OMC-3 cells. Confluent monolayers of tumor cells were cultured for 48 h with or without 0.1–10 μM E_2 and Prog or 0.1–10 nM EGF and TGF-α in serum-free Ham's F-12. TCM samples were applied to fibrinogen-embedded SDS-polyacrylamide gels, and active enzymes were detected as unstained bands, as described in Fig. 3.

Table I. Effects of Sex Steroids and Growth Factors on dThdPase Expression of OMC-3 Cells

Sex steroid (μM) or growth factor (nM)		dThdPase (unit/mg protein)	Ratio[a)]
	Control	8.2	1
E_2	0.1	7.8	0.95
	1	7.8	0.95
	10	8.1	0.99
Prog	0.1	9.1	1.11
	1	9.0	1.10
	10	8.5	1.04
EGF	0.1	9.0	1.10
	1	10.7	1.30
	10	11.5	1.40
TGF-α	0.1	11.6	1.41
	1	12.8	1.56
	10	14.2	1.73

a) Relative expression compared with control values.

Table II. Effects of Sex Steroids and Growth Factors on the Sensitivity of OMC-3 Cells to 5′-dFUrd and 5-FUra

Sex steroid (μM) or growth factor (nM)		Sensitivity to[a)]			
		5′-dFUrd		5-FUra	
		IC_{50} (μg/ml)	Ratio[b)]	IC_{50} (μg/ml)	Ratio[b)]
	Control	56.0	1	11.0	1
E_2	1	54.0	1.04	10.6	1.04
	10	56.4	0.99	11.2	0.98
Prog	1	58.0	0.97	11.0	1.00
	10	56.6	0.99	11.4	0.96
EGF	1	38.0[c)]	1.47	9.8	1.12
	10	10.8[c)]	5.19	7.7	1.43
TGF-α	1	26.0[c)]	2.15	8.6	1.28
	10	8.6[c)]	6.51	7.4	1.49

a) Mean of triplicates.
b) Relative sensitivity compared with control values.
c) $P<0.05$ compared with control values.

and TGF-α stimulated the expression of 72 kDa type IV collagenase, stromelysin and uPA. Moreover, we examined the effects of sex steroids and growth factors on the proliferative activity of OMC-3 cells by MTT assay as previously described.[25)] Cells (1.0×10^4/well) were uniformly seeded into 96-well microplates and incubated at 37°C for 24 h. After having been washed twice with serum-free Ham's F-12 with 0.1% BSA, the cells were cultured for another 24 h in the same medium. The medium was then removed and replaced with serum-free Ham's F-12 with 0.1% BSA containing 0.1–10 μM E_2 and Prog or 0.1–10 nM EGF and TGF-α. After further incubation at 37°C for 24 or 48 h, MTT was added to each well and the absorbance was evaluated as described above. The proliferative activity of tumor cells was not affected by sex steroids or growth factors within 48-h incubation (data not shown). Therefore, it is likely that the stimulatory effects of EGF and TGF-α on the invasive activity of OMC-3 cells are associated with tumor cell motility and the degradative cascade of ECM components by proteinases. EGF and TGF-α are assumed to act through a common receptor and to share many biological activities.[34)] Their stimulatory effects on the invasive activity of OMC-3 cells may be mediated by EGFR on the cellular membrane, as indicated by Scatchard plot analysis. On the other hand, E_2 and Prog did not affect proteinase expression, which may be due to the absence of ER and PR on the cells. Therefore, the inhibitory effect of Prog on tumor cell invasion was considered to depend on its inhibitory action on the motility of tumor cells, which might be associated with its pharmacological action not mediated by PR. We previously demonstrated that medroxyprogesterone acetate (MPA) did not have any effect on the expression of type IV collagenases or uPA in endometrial adenocarcinoma SNG-M cells, though, 1–10 μM MPA significantly inhibited migration and invasion of the cells. The inhibitory effect of MPA on the motility of SNG-M cells was also confirmed by wound assay.[9, 25)] Synthetic progestins at high concentrations might act as negative regulators of the invasion process of cultured gynecological cancer cells, possibly via their inhibitory effects on the motility of tumor cells. To clarify the molecular events in the biological action of Prog and MPA on the motility of tumor cells, their direct effects on the cytoskeletal structure of tumor cells should be further examined.

Inflammatory cytokines and factors, such as TNFα, IL-1α and INFγ, that up-regulate the expression of type IV collagenase and PyNPase, have been detected in various human and mouse tumor tissues.[11, 12)] Moreover, some of these factors have been reported to increase the metastatic potential of tumor cells.[15–17)] These tumor environmental factors would enhance the metastatic ability of tumor cells by up-regulating MMP activity, and 5′-dFUrd would inhibit metastasis by selectively killing such tumor cells because of their high PyNPase activity.[23)] The present results revealed that EGF and TGF-α simultaneously up-regulated invasive activity, proteolytic enzyme level and dThdPase expression of OMC-3 cells, and enhanced the antiproliferative action of 5′-dFUrd. In contrast, the sensitivity of tumor cells to 5-FUra was increased to a slight extent in the presence of these growth factors. This preferential increase in the sensitivity of tumor cells to 5′-dFUrd may be due to the induction of dThdPase, because conversion of 5′-dFUrd to 5-FUra can be accelerated by this enzyme. 5-FUra is further anabolized to 2′-deoxy-5-

fluorouridine by dThdPase and then to the active metabolite 5′-fluorodeoxyuridine monophosphate by thymidine kinase.[22] Therefore, the slight increase in 5-FUra sensitivity should also be due to the dThdPase induction. However, E_2 and Prog did not have any significant effect on proteinase and dThdPase expression, or 5′-dFUrd sensitivity of the cells. Only EGF and TGF-α would act as regulatory factors on the degradative cascade of ECM components in ovarian adenocarcinoma cells negative for hormone receptors.

In conclusion, we have demonstrated that EGF and TGF-α simultaneously up-regulate the potential of OMC-3 cells to invade extracellular matrices and their dThdPase expression, which are subsequently associated with the specific action of 5′-dFUrd in selectively killing ovarian adenocarcinoma cells with high invasive and metastatic potential.

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