Article

Regulatory Effect of E2, IL-6 and IL-8 on the Growth of Epithelial Ovarian Cancer Cells

Yue Wang[1], Jie Yang[1], Yan Gao[1, 2], Yongrui Du[1], Leyuan Bao[1], Wenyan Niu[1] and Zhi Yao[1, 3]

To determine the regulatory effects of estrogen and cytokine IL-6 and IL-8 on the growth of epithelial ovarian cancer (OVCA), we first examined the status of estrogen receptors (ERα and ERβ), IL-6 receptor (IL-6Rα and gp130), and IL-8 receptor (IL-8RA and IL-8RB) on five epithelial OVCA cell lines by semiquantitative RT-PCR and Western blot analysis. Results showed that the expressions of these receptors were variable on the five cells. Those OVCA cells expressing the receptors were selected to study related molecular mechanism. MTT assay was performed to observe the effects of 17β-estradiol (E2), IL-6 and IL-8 on cell proliferation. We discovered that E2 markedly promoted the proliferation of CAOV-3 and OVCAR-3 cell in a time- and dose-dependent manner. Tamoxifen (Txf), an ER inhibitor, completely blocked the proliferation of the E2-induced cells, and IL-6- or/and IL-8-neutralizing antibody only showed partially blocking activity. IL-6 and IL-8 were able to significantly stimulate CAOV-3 and OVCAR-3 cell proliferation in a time- and dose-dependent manner, which had a potential synergistic effect on CAOV-3 cells but not on OVCAR-3 cells. The cell proliferation induced by these two cytokines was abolished completely by their specific neutralizing antibodies, partially by Txf, but not by unrelated goat IgG. Taken together, our results suggested that estrogen, IL-6 and IL-8 could modulate OVCA growth by forming a reciprocal cascade with amplifying effect. *Cellular & Molecular Immunology*. 2005;2(5):365-372.

Key Words: estrogen, IL-6, IL-8, cell proliferation, epithelial ovarian cancer

Introduction

Ovarian cancer (OVCA) is the second most common and the most deadly malignancy of the female reproductive tract. More than 90% of OVCAs arise from the single layer of human ovarian surface epithelial cells covering the ovary. Etiological factors involved in ovarian carcinogenesis remain poorly defined, and effective treatment protocols are limited (1, 2). Epidemiologic data suggest that gonadotropins and sex steroid hormones, especially estrogens, are risk factors for epithelial ovarian cancer.

IL-6 is a pleiotropic cytokine and plays important roles in cell proliferation, cell differentiation, immune defense, and hematopoiesis (3-5). It is also involved in the malignant transformation and progression of various cancers, including renal-cell, prostate, lung, breast carcinomas, leukemia, as well as Kaposi's sarcoma (6-12). IL-8 is a CXC chemokine, initially identified as a regulator for the recruitment and trafficking of leukocytes to sites of inflammation (13, 14). Subsequent studies demonstrated that IL-8 is a common chemotactic factor, implicated in the metastasis and angiogenesis of a variety of cancers. It has been widely reported that IL-6 and IL-8 are overexpressed in epithelial OVCA (15-17), suggesting these two cytokines are likely involved in the progression of OVCA.

In the present study, we first investigated the status of ERα, ERβ, IL-6Rα, gp130, IL-8RA, and IL-8RB on five OVCA cell lines and then observed the effect of 17β-estradiol (E2), IL-6, and IL-8 on cell proliferation *in vitro*, and investigated the potential reciprocal regulation of estrogen and these two cytokines in the growth of epithelial OVCA. Our work provides an important advance in understanding the molecular mechanism of the interaction between estrogen and cytokines in the genesis and development of epithelial OVCA.

Materials and Methods

Cell lines and cell culture

Human OVCA cell lines OVCAR-3, CAOV-3, and SKOV-3

[1]Department of Immunology, Tianjin Medical University, Tianjin 300070, China;

[2]Department of Gynecologic Oncology, Tianjin Women's Hospital, Tianjin 300070, China;

[3]Corresponding to: Dr. Zhi Yao, Department of Immunology, Tianjin Medical University, 22 Qixiangtai Road, Tianjin 300070, China. Tel: +86-22-235-42817, E-mail: yaozhi@tmu.cn.

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were obtained from the American Type Culture Collection. HO-8910PM high-metastatic human OVCA cell line and the parental low-metastatic HO-8910 cell line were established by the Zhejiang Cancer Research Institute, and purchased from Shanghai Institute of Cell Biology, Chinese Academy of Sciences. CAOV-3, HO-8910, and HO-8910PM cells were cultured in RPMI 1640 (Life Technologies) containing 10% fetal bovine serum (FBS) (Hyclone), and OVCAR-3 cells were grown in RPMI 1640 with 20% FBS and 155 U/ml insulin, and CAOV-3 cells were grown in DMEM (Life Technologies) with 15% FBS. These culture media were supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin.

Semiquantitative reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated from five OVCA cells using TRIzol reagents (Life Technologies) according to the manufacturer's instructions and quantified by UV absorbance at 260-280 nm. Sequences of the primers were designed by Oligo 6 software and synthesized by TaKaRa Biotechnology (Dalian) Co., Ltd. Primers for ERα , 5'-CGG CTC CGC AAA TGC TAC GAA GTG-3'(forward) and 5'-AGC GCC AGA CGA GAC CAA TCA TCA-3'(reverse) (18), for ERβ, 5'-GAG CAC GGC TCC ATA TAC AT-3' (forward) and 5'-GCC TTA CAT CCT TCA CAC GA-3'(reverse) and for GAPDH, 5'-CTC AGA CAC CAT GGG GAA GGT GA-3'(forward) and 5'-ATG ATC TTG AGG CTG TTG TCA TA-3' (reverse). One Step RNA PCR Kit (AMV) (TaKaRa Biotechnology) to thermal cycling as follows: one cycle at 50°C for 15 min; at 94°C for 2 min with an additional 34 cycles at 94°C for 30 s; at 61°C (ERα) or 50°C (ERβ) for 30 s; at 72°C for 2 min; and 5 min at 72°C for the final extension. PCR products were fractionated on a 1.5% agarose gel and bands were analyzed with Quantity One-4.5.6 software (Bio-Rad). Target fragment levels were normalized against GADPH, and data are presented as target mRNA/GADPH ratio.

Western blot analysis

The cells were harvested when 80% confluent and seeded in 100-mm culture dishes with the density of 5×10^6 cells. Cells were washed twice with ice-cold PBS and lysed in ice-cold RIPA buffer. The extracts were placed on ice for 45 min and centrifuged to remove cellular debris. The protein content of the supernatants was determined using the bicinchoninic acid assay kit (Pierce Biochemicals). Fifty μg of protein was run on 8-10% SDS-PAGE gels and electrotransferred to PVDF membranes. The membranes were blocked with 5% nonfat dry milk in Tris-buffered saline containing 0.05% Tween overnight at 4°C, and then probed with rabbit polyclonal antibodies for ERα (1:400), ERβ (1:400), IL-6Rα (1:200), gp130 (1:500), IL-8RA (1:400), and IL-8RB (1:400) (Santa Cruz Biotechnology), respectively, at room temperature for 3 h, followed by horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (1:5,000, KPL), and visualized by chemiluminescent substrate kit (SuperSignal® Westpico Trial Kit, Pierce Biochemicals). Those membranes were subsequently stripped by incubating with stripping buffer at 50°C for 30 min and then reprobed with a mouse monoclonal antibody for β-actin at 1:4,000 dilution (Sigma) with horseradish peroxidase-conjugated goat anti-mouse secondary antibody (1:5,000 dilution, KPL) and visualized by chemiluminescent substrate kit. Relative band intensities and areas were assessed by densitometric analysis (Scion Image Analysis software program). Target protein levels were normalized against β-actin to control for variance in sample loading and transfer.

Effect of E2, IL-6, and IL-8 on OVCA cell proliferation

CAOV-3 and OVCAR-3 cells were cultured in 96-well plates at 2×10^4 cells per well, respectively, for 24 h, and then switched to medium containing 5% FBS and cultured for another 24 h. The cells were treated daily with 0.1, 1, 10, 100, or 1000 nM E2 (Sigma-Aldrich, St. Louis, MO) in ethanol, or 10, 25, or 50 ng/ml recombinant human IL-6 and IL-8 (R&D Systems, Minneapolis, MN) in PBS containing 0.1% bovine serum albumin (BSA). The final ethanol concentration was 0.1%. Control cells were treated daily with corresponding vehicle, respectively. After 5 days (in CAOV-3 cells) or 6 days (in OVCAR-3 cells which grow relatively slowly) treatment, the cells were incubated in 100 μl of MTT solution (0.5 mg/ml, Sigma) for 4 h at 37°C. After centrifugation, 100 μl of 0.04 N HCl-isopropanol was added to each well so as to dissolve purple formazan crystal, then the absorbance of each well was measured by using an ELISA microplate reader at 490 nm.

Blocking E2/cytokine-induced OVCA cell proliferation

To ascertain whether the observed E2/cytokine-induced cell proliferation is mediated through a ER pathway, cells were pretreated with 100 nM tamoxifen (Txf, Sigma), an ER antagonist (19) for 30 min, then treated with 10 nM E2, 50 ng/ml IL-6 or 10 ng/ml IL-8, respectively. To neutralize E2-induced expression of IL-6 or/and IL-8, CAOV-3 cells were cultured with 10 nM E2 and different doses (1, 5, 10 μg/ml) of IL-6-neutralizing antibody (AB-206-NA, R&D) or/and IL-8-neutralizing antibody (AB-206-NA, R&D) or 10 μg/ml isotype goat anti-human IgG. To further investigate the specificity of IL-6 and IL-8 in the observed growth stimulation of OVCA cells, CAOV-3 cells were cultured with 50 ng/ml IL-6 or 10 ng/ml IL-8 and 10 μg/ml their respective neutralizing antibody or isotype goat anti-human IgG. To ensure stable availability, estrogen, ER antagonist, cytokines, and cytokine-neutralizing antibodies were added to cells daily. Cell proliferation was measured with MTT assay, as described above.

Statistical analysis

Data are indicated as the mean of two to three experiments, each in triplicate or sextuple samples for individual treatments or dosage regimens. Statistical analysis was carried out using a one-way ANOVA, followed by Tukey's *post hoc* test. Values are presented as the mean ± SD. All statistical tests were two-sided and were considered to be

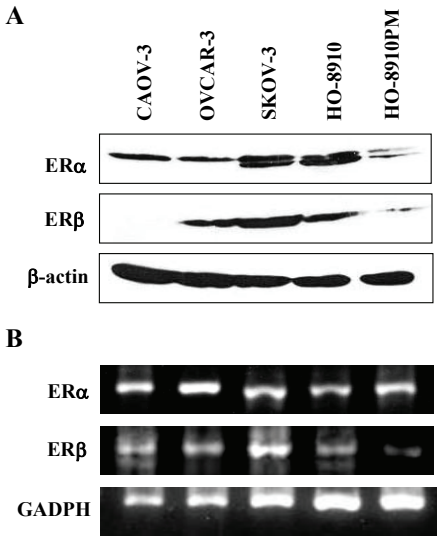

Figure 1. Protein and mRNA levels of ERα and ERβ on five OVCA cell lines. (A) Detection of ERα protein and ERβ protein by Western blot analysis. (B) Detection of ERα mRNA and ERβ mRNA by RT-PCR. The experiment shown was the representative from two separate experiment results.

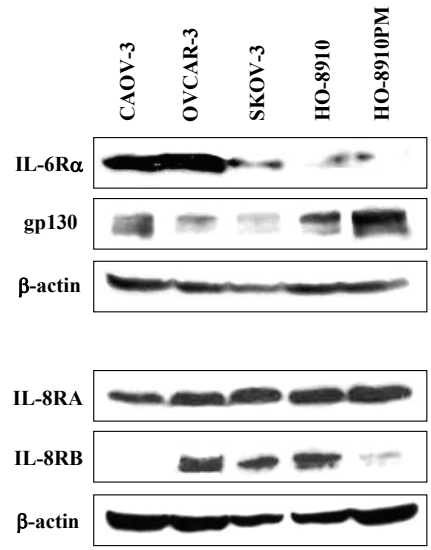

Figure 2. Detection of protein levels of IL-6 receptor (IL-6Rα and gp130) and IL-8 receptor (IL-8RA and IL-8RB) in five OVCA cell lines by Western blot analysis. The experiment shown was the representative from two independent experiment results.

statistically significant at $p < 0.05$.

Results

Expression of ERα and ERβ protein and mRNA in five OVCA cell lines

To investigate the effect of E2 on OVCA cells, we first determined the expression of ERα and ERβ on five OVCA cell lines by Western blot analysis and semiquantitative RT-PCR. As shown in Figure 1A, high levels of ERα protein (68 kD) were observed in CAOV-3 and SKOV-3 cells, moderate levels were found in OVCAR-3 and HO-8910 cells, and they were approximately 2.4-fold and 1.9-fold, respectively, of the level detected in HO-8910PM cells. In addition to the predicted 68 kD protein band, an additional protein band of a smaller size was present in SKOV-3, HO-8910, and HO-8910PM cells. High level of ERβ protein (55 kD) was detected in SKOV-3 cells, moderate levels were found in OVCAR-3 and HO-8910 cells, and they were approximately 3.3-fold and 2.2-fold, respectively, of the level in HO-8910PM cells. It was noted that no ERβ protein was detected in CAOV-3 cells.

Transcript levels of ERα and ERβ in five OVCA cells are shown in Figure 1B. ERα mRNA expressions in CAOV-3 and OVCAR-3 cells were strong in the presence of 1 μg RNA, whereas SKOV-3, HO-8910, and HO-8910PM cells gave weaker signals than CAOV-3 and OVCAR-3 cells and smaller than expected size in the presence of 2 μg RNA instead of 1μg RNA. In parallel with protein level, high, moderate, and low levels of ERβ mRNA were observed in SKOV-3, OVCAR-3 and HO-8910, and HO-8910PM cells. Paradoxically, CAOV-3 cells also expressed moderate level of ERβ mRNA.

Expression of IL-6Rα, gp130, IL-8RA, and IL-8RB protein in five OVCA cell lines

To investigate the effect of IL-6 and IL-8 on OVCA cells, we also determined whether these cell lines express IL-6Rα , gp130, IL-8RA, and IL-8RB. The presence of these receptors was assessed by Western blot analysis. As shown in Figure 2A, expression levels of IL-6Rα protein (80 kD) were 3.6-fold, 3-fold, 2.5-fold, and 1.5-fold higher in OVCAR-3, CAOV-3, SKOV-3, and HO-8910 cells, respectively, than that in HO-8910PM cells. Significant increases (3.2-fold, 1.7-fold, and 1.4-fold, respectively) of gp130 protein (130 kD) were observed in HO-8910PM, HO-8910, and CAOV-3 cells, respectively, compared with SKOV-3 and OVCAR-3 cells which expressed similar low level of gp130 protein (Figure 2A). As shown in Figure 2B, the expression level of IL-8RA protein (60 kD) in SKOV-3 cells is similar to that in HO-8910 cells. IL-8RA protein levels in these two cells, as well as HO-8910PM and OVCAR-3 cells were 2.3-fold,

Table 1. Expression of protein levels for estrogen receptor (ERα and ERβ), IL-6 receptor (IL-6Rα and gp130), and IL-8 receptor (IL-8RA and IL-8RB) in CAOV-3 and OVCAR-3 cells by Western blot analysis

Cell lines	ERα (68 kD)	ERβ (55 kD)	IL-6Rα (80 kD)	gp130 (130 kD)	IL-8RA (60 kD)	IL-8RB (46 kD)
CAOV-3	1.4	-	1	1.4	1	-
OVCAR-3	1	+	1.2	1	1.5	+

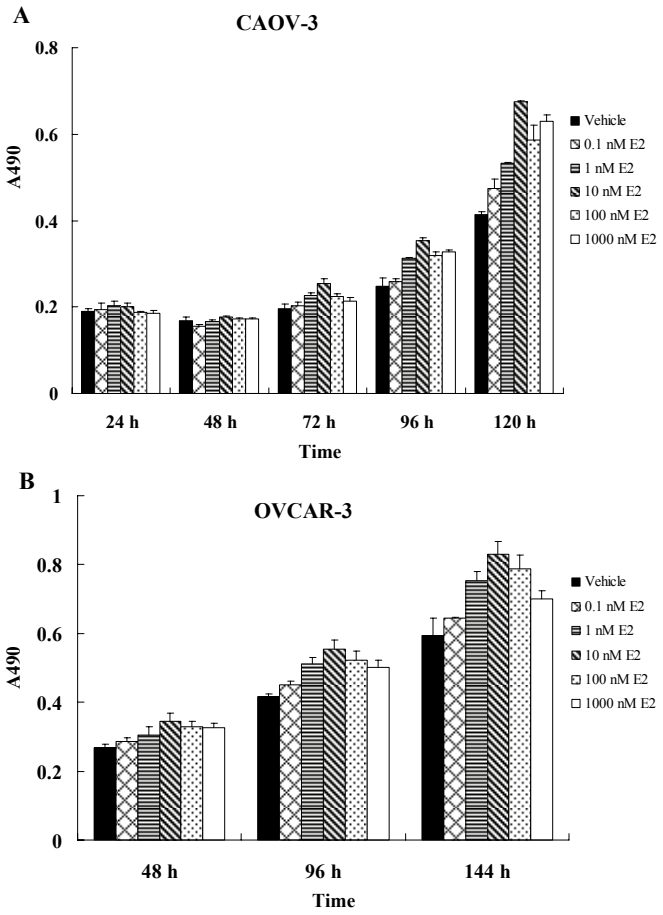

Figure 3. Effect of E2 on the growth of OVCA cell lines CAOV-3 (A) and OVCAR-3 (B) by MTT assay. Values are indicated as the percentage of growth compared with the control value and are the mean ± SD of two experiments with sextuple samples.

1.8-fold and 1.5-fold, respectively, of measured in CAOV-3 cells. Significant increases (6.6-fold, 6.1-fold, and 4.7-fold, respectively) of IL-8RB protein (46 kD) were found in HO-8910, OVCAR-3, and SKOV-3 cells, respectively, compared with HO-8910PM cells, whereas no IL-8RB protein was detected in CAOV-3 cells (Figure 2B).

Effect of E2, IL-6, and IL-8 on OVCA cell proliferation

Based on the above results, the CAOV-3 and OVCAR-3 cells were used for studying the effects of E2, IL-6, and IL-8 on cell proliferation. Results demonstrated that E2, IL-6, and IL-8 may stimulate OVCA cell growth in a time- and dose-dependent manner. In CAOV-3 cells, the growth stimulation was undetected till over 72 h E2 treatment ($p < 0.05$), with maximum increase (130%, 143%, and 162% of control, respectively) at 10 nM (Figure 3A). Treatment with E2 for 48 h, 96 h, or 144 h resulted in a significant increase in OVCAR-3 cell counts ($p < 0.05$), with maximum increases (128%, 133%, and 140% of control, respectively) at 10 nM (Figure 3B). E2-promoted cell proliferation in CAOV-3 cells appeared to be more significant than that in OVCAR-3 cells. After stimulation of IL-6 at the dose of 25 ng/ml for 120 h, IL-6-promoted cell proliferation was the most apparent in CAOV-3 (161% increase) and at the dose of 50 ng/ml for 96 h in OVCAR-3 cells (250% increase) (Figures 4A and 4B). After stimulation of IL-8 at the dose of 25 ng/ml for 120 h, IL-8-promoted cell proliferation was the most apparent in CAOV-3 (149% increase) and at the dose of 10 ng/ml for 96 h in OVCAR-3 cells (173% increase) (Figure 4A and 4B). Cell proliferation stimulated by IL-6 or IL-8 in CAOV-3 cells appeared to be less significant than that in OVCAR-3 cells. Considering that both IL-6 and IL-8 may stimulate cell growth, we reasoned whether both had a potential synergistic effect. As shown in Figures 4A and 4B, IL-6 and IL-8 had a potential synergistic effect of promoting cell growth at the dose of 10 ng/ml in CAOV-3 cell, but not in OVCAR-3 cells.

Blocking E2- and cytokines-induced OVCA cell proliferation

To investigate whether the observed E2-stimulated cell proliferation is mediated through a ER pathway, as well as to neutralize E2-induced IL-6 or/and IL-8 in the observed growth stimulation, CAOV-3 cells were cultured for 4 days in the presence of 10 nM E2 and 100 nM tamoxifen or different doses (1, 5, 10 μg/ml) of IL-6-neutralizing antibody or/and IL-8-neutralizing antibody or 10 μg/ml isotype goat anti-human IgG. As shown in Figure 5A, tamoxifen, an ER antagonist, completely abolished E2-induced cell growth ($p < 0.01$), indicating that estrogen induces OVCA cell growth through the ER. IL-6- or/and IL-8-neutralizing antibody (10 μg/ml) partially inhibited E2-induced cell growth ($p < 0.05$), but both all failed to reduce the cell proliferation of vehicle control ($p > 0.05$). No dramatic difference in blocking E2-induced cell growth was discovered between both alone and together ($p > 0.05$), and isotype goat IgG had no effect ($p > 0.05$). These results suggest that estrogen promotes OVCA cell growth at least in part through induction of IL-6 and IL-8.

Mechanistically, there are two ways by which IL-6 or IL-8 can promote OVCA cell growth in the absence of estrogen. IL-6 or IL-8 can activate a growth pathway that completely bypasses estrogen and ER activation. Alternatively, IL-6 or IL-8 can activate the ER in the absence of estrogen, and utilize the existing ER pathway to stimulate cell proliferation. To ascertain the specificity of IL-6 and IL-8 and the involvement of ER in the observed growth stimulation of OVCA cells, CAOV-3 cells were cultured for 4 days in the presence of 50 ng/ml IL-6 or 10 ng/ml IL-8 and 10 μg/ml of respective neutralizing antibody or isotype goat anti-human IgG or 100 nM tamoxifen. As illustrated in Figure 5B, IL-6-/IL-8-induced CAOV-3 cell proliferation was completely prevented by respective specific neutralizing antibodies ($p < 0.05$), and not by isotype goat IgG ($p > 0.05$), demonstrating the growth stimulation observed in IL-6- or IL-8-treated OVCA cells is IL-6 or IL-8 specific. Tamoxifen also partially reduced IL-6-/IL-8-promoted OVCA cell proliferation ($p < 0.05$), suggesting that IL-6 and IL-8 may stimulate OVCA cells growth through the ER.

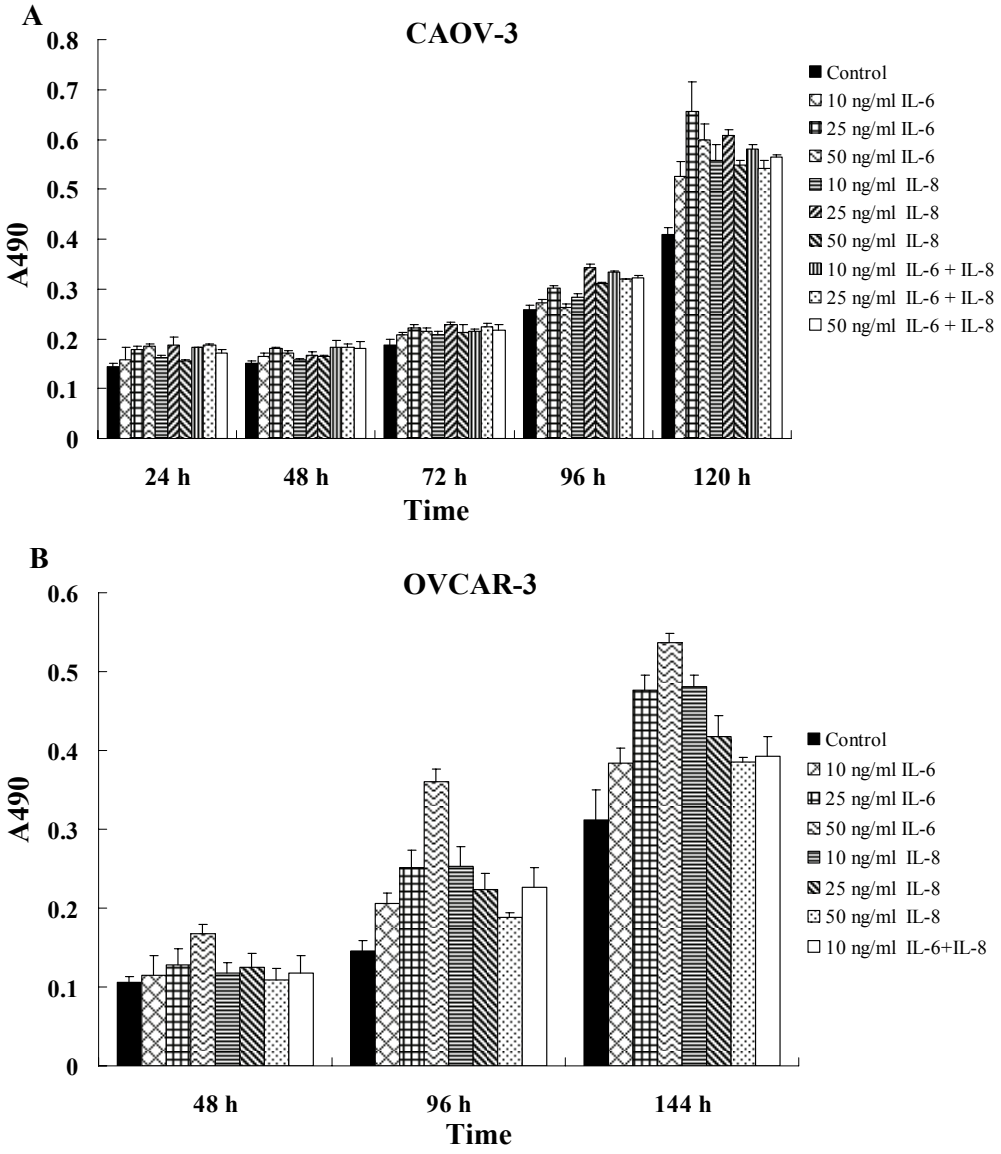

Figure 4. Synergistic effect of IL-6 and IL-8 on the growth of OVCA cell lines CAOV-3 (A) and OVCAR-3 (B) by MTT assay. Values are expressed as the percentage of growth compared with the control value and are the mean ± SD of two experiments with sextuple samples.

Discussion

Epidemiological data suggest that estrogens may play important roles in the genesis and progression of epithelial OVCA. However, the molecular mechanism remains exclusive. Biological effects of estrogens are mediated through interactions with their intracellular receptors (20). Recent studies have revealed different tissue distributions and expression levels of ERα and ERβ in the ovary, suggesting different biological roles of ERα and ERβ in these tissues (21, 22). In the present study, we demonstrated protein and mRNA levels of ERα and ERβ on five OVCA cell lines were variable. In addition to the predicted 68 kD protein band of ERα , an additional protein band of a smaller size was found in SKOV-3, HO-8910, and HO-8910PM cells. Correspondingly, the size of the ERα mRNA was smaller than the expected one. In parallel with protein level, an additional much fainter transcript product of a smaller size was also present in these three cells. Previously, it has reported the coexpression of ERα wild-type transcript and its variant deletion in exon 2 in SKOV-3 cells (23). In this study, we found that HO-8910 and HO-8910PM cells also co-expressed ERα mRNA and its variant form by Western blot and RT-PCR analyses. Therefore, we hypothesis that the deletion mutant is also present in these two cell lines, however, it remains to be further determined.

Our observation that estrogen did not affect SKOV-3, HO-8910, and HO-8910PM cell proliferation *in vitro* (data not shown) was consistent with previous reports (24, 25). It is likely that estrogen resistance in these cell lines is due to abnormal expression of the ERα. However, estrogen may markedly promote CAOV-3 and OVCAR-3 cell proliferations in a time- and dose-dependent manner, for these two cells express functional ERα. Cell proliferation stimulated by E2

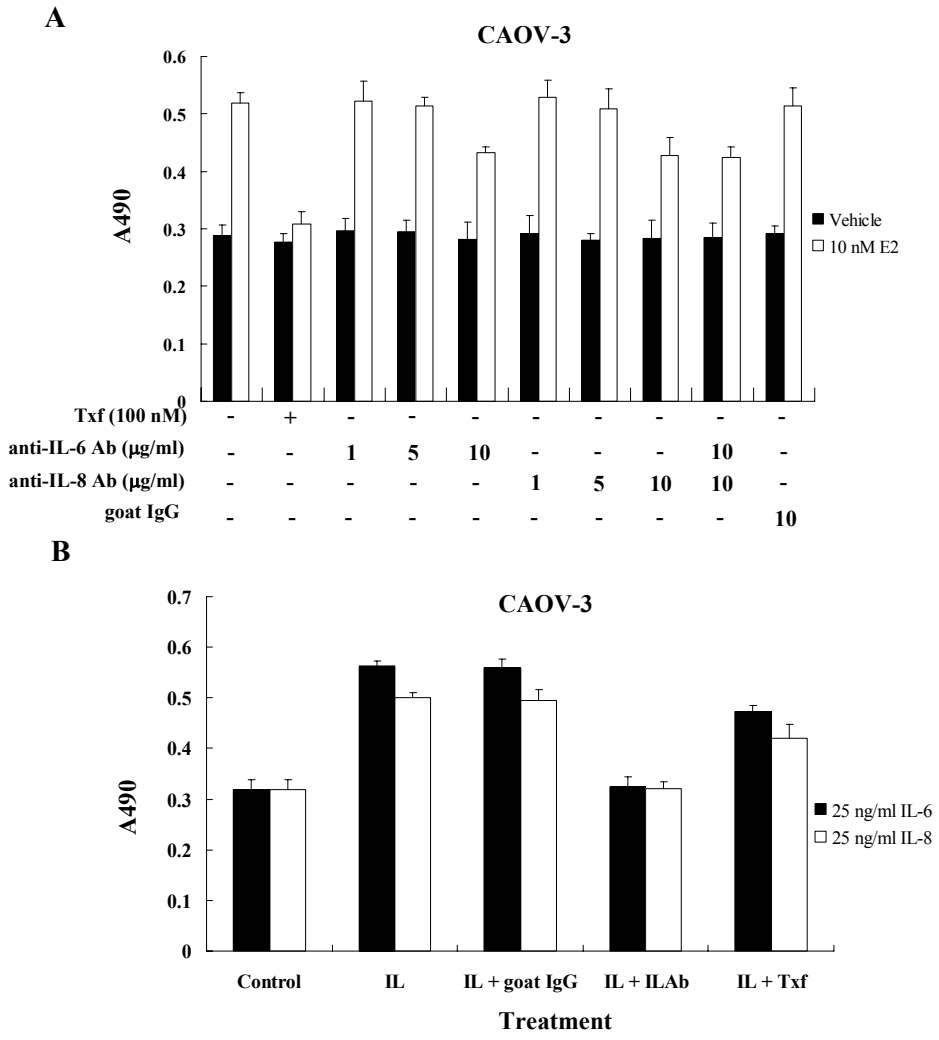

Figure 5. Blocking E2- and cytokine-induced OVCA cell proliferation by MTT assay. (A) Txf (100 nM), an ER antagonist was added before treatment with 10 nM E2. To neutralize E2-induced IL-6 or/and IL-8, cells were cultured with E2 and different doses of IL-6 or/and IL-8-neutralizing antibody (1, 5, 10 μg /ml) or 10 μg/ml isotype goat anti-human IgG. (B) Cells were cultured with 50 ng/ml IL-6 or 10 ng/ml IL-8 and 10 μg /ml their respective neutralizing antibody or isotype goat anti-human IgG. Txf (100 nM) was added 30 min before treatment with IL-6 or IL-8.

in CAOV-3 cells appeared to be more significant than that in OVCAR-3 cells. ERα protein level was approximately 1.4-fold higher in CAOV-3 cells than that in OVCAR-3 cells, whereas no ERβ protein was detected in CAOV-3 cells. Therefore, the response to estrogen could largely depend on the ERα expression in quality and quantity but not ERβ. Using Western blot analysis, expression of ERβ protein in CAOV-3 cells was undetected in the presence of 50 μg protein. However, moderate expression of ERβ mRNA in CAOV-3 cells, which was in agreement with the finding of Lau et al. (23), was observed in the presence of 1 μg RNA by using semiquantitative RT-PCR. The difference in ERβ at transcriptional and translational levels may be explained by difference in transcript and translation levels of ERβ gene itself in CAOV-3 cells and/or perhaps difference in sensitivity of approaches.

It has been widely reported that IL-6 and IL-8 are overexpressed in epithelial OVCA, and high level of IL-6 correlated with a poor final outcome, and increased IL-6 and IL-8 correlated with a poor initial response to chemotherapy (15-17). It was reported that most of OVCA cell lines constitutively express IL-6 and IL-8 (26). The levels of spontaneous secretion of IL-6 and IL-8 are apparent variable in five OVCA cell lines tested in our previous study. These reports support that these two cytokines are likely involved in the progression of OVCA. In this study, we demonstrated that IL-6 and IL-8 may promote cell proliferation of CAOV-3 and OVCAR-3 cells in a time- and dose-dependent manner. Cell proliferation stimulated by IL-6 and IL-8 in CAOV-3 cells appeared to be less significant than that in OVCAR-3 cells. Variability of expression levels of cytokine receptors on these two cell lines is likely to account for such a response. Semiquantitative analysis showed that the expression of IL-6Rα and IL-8RA were approximately 1.2-fold and 1.5-fold, respectively, higher on OVCAR-3 cells than on CAOV-3 cells, whereas that of gp130 was approximately 1.4-fold higher on CAOV-3 cells than on OVCAR-3 cells, and no IL-8RB protein was observed on CAOV-3 cells, suggesting that the response to IL-6 may largely depend on the expression of IL-6Rα (IL-6-specific receptor subunit) but not on gp130 (a signal transducer, that is used by other

cytokine receptors), whereas the response to IL-8 could depend on the expressions of IL-8RA and IL-8RB, especially IL-8RB which, unlike IL-8RA, is not specific for IL-8 and can bind to other chemokines such as growth-related oncogenne α, but has higher affinity for IL-8 than IL-8RA (27). The observation that IL-6 and IL-8 had a potential synergistic effect on CAOV-3 cell but not on OVCAR-3 cell could be associated with variable expressions of cytokine receptors in these two cell lines.

The precise mechanism responsible for estrogen-stimulated OVCA cell growth remains unclear. The observation that tamoxifen, an ER antagonist, completely abolished E2-induced CAOV-3 cell proliferation suggests that E2 stimulated OVCA cell proliferation through the ER. It remains to be determined which type of receptor mediates the effect of estrogen in these cells. Other studies have demonstrated that homodimers ERα/ERα and ERβ/ERβ or heterodimers ERα/ERβ can be formed *in vitro*, bind to the estrogen response elements, and stimulate the transcription of a reporter gene (28, 29). Therefore, the relative expression of ERα and ERβ and the difference in DNA-binding activity between heterodimers and homodimers could determine the tissue specific effects of estrogen action.

In this study, we demonstrated that estrogen-induced cell proliferation was partially inhibited by IL-6- or/and IL-8-neutralizing antibody, suggesting that the estrogen-induced increase in cell proliferation is mediated partially by IL-6 and IL-8, although other growth factors may also make contribution. Despite different OVCA cell lines used in the study, our partial findings are similar to other labs (30, 31), who demonstrated estrogen (estrone and E2) stimulated cell proliferation and IL-6 expression on OVCA 429 and OVCA432 cells, and estrogen-induced the proliferation of OVCA cells could be partially blocked by IL-6-neutralizing antibody. Furthermore, our study provides the first evidence that estrogen-stimulate cell proliferation is also mediated by IL-8. Our data suggested that a possible mechnism through which estrogen stimulates the growth of OVCA by the ER pathway and at least in part through induction of IL-6 and IL-8.

ER modulated a diverse array of intracellular signal transduction cascades. In recent years, increasing evidence (32, 33) has suggested that the actions of estrogens at target tissues can be divided into long-term "genomic" actions that are mediated by intracellular ER-induced changes in gene expression, and rapid or short term "nongenomic" actions that are mediated by plasma membrane. The cross-talk between membrane ER-induced intracellular signal transduction cascades, and IL-6-/IL-8-induced signal transduction pathways may include Ras/Raf/MEK/MAPK, PI-3-kinase, and G protein-mediated signaling pathways. In the present study, we demonstrated that IL-6- or/and IL-8-neutralizing antibody could completely block IL-6- or IL-8-induced cell growth and partially block estrogen-induced cell growth, suggesting the estrogen-induced cell growth is, at least in part, due to regulate expression of IL-6 and IL-8 through common intracellular signaling pathways. Is there alternative pathway, the existing ER pathway, utilized by IL-6 and IL-8 to induce growth of OVCA cells in addition to their respective receptor pathway? We demon- strated that tamoxifen, an ER antagonist, partially reduced IL-6-/IL-8-promoted OVCA cell proliferation, suggesting that IL-6 and IL-8 may stimulate OVCA cell growth through the ER.

Taken together, our results suggested that estrogen, IL-6, and IL-8, modulated reciprocally to form jointly amplifying effect cascade on OVCA growth. Our study provides an important advance in understanding the molecular mechanism of the interaction between estrogen and cytokines in the genesis and development of epithelial OVCA.

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