Role of Estrogen and Progesterone in the Survival of Ovarian Tumors — A Study of the Human Ovarian Adenocarcinoma Cell Line OC-117-VGH

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Background: The role of estrogen and progesterone in ovarian carcinogenesis and the growth and survival of ovarian cancer cells is controversial. In this study, we tested the effects of various concentrations of estrogen and progesterone on the survival of an ovarian cancer cell line.

Methods: The ovarian adenocarcinoma cell line, OC-117-VGH, is deficient in the following receptors: estrogen receptor (ER)α, ERβ, and progesterone receptor (PR). Serial concentrations of estrogen and progesterone were used to evaluate the effects of estrogen and progesterone on the survival of ovarian cancer cells. The apoptosis-related genes *Bcl-2* and *Bax* were used to check the possible mechanism of an estrogen or progesterone effect on survival of the cancer cell line.

Results: Estrogen 0.01–1.0 μM and progesterone 0.1–10.0 μM affected cell survival. As predicted, progesterone successfully downregulated anti-apoptotic *Bcl-2* expression, and dose-dependently suppressed *Bcl-2* expression in tumor cells. Paradoxically, estrogen showed the same effects. In addition, both hormones downregulated pro-apoptotic *Bax* expression. The net effect confirmed the role of downregulated *Bcl-2* in reducing the survival of ovarian cancer cells.

Conclusion: Based on the findings of decreased survival and/or growth in OC-117-VGH ovarian adenocarcinoma cells treated with either estrogen or progesterone, we suspect that both hormones act effectively against ER-negative and PR-negative ovarian cancer cells. These findings should lead to a reassessment of hormone therapy for ovarian cancers. [*J Chin Med Assoc* 2005;68(8):360–367]

Key Words: apoptosis, cell line, estrogen, ovarian cancer, progesterone

Introduction

Ovarian cancer is a malignant disease of worldwide importance, and is the leading cause of cancer mortality in female patients, not only in the US, but also in Taiwan.[1,2] The initial treatment for ovarian cancer is surgical resection of the primary tumor, followed by postoperative adjuvant chemotherapy or possible radiation.[3] Ovarian tumors showing resistance to chemotherapy usually suggest a poor prognosis.[4] The causes of ovarian epithelial cancer have not yet been clearly defined, but recent epidemiologic studies reported that estrogen and/or progesterone may promote ovarian tumor progression in postmenopausal women.[5] In addition, the Women's Health Initiative (WHI) randomized trial expressed concerns about a possible association between gynecologic malignancies and postmenopausal hormone therapy.[6] There is also growing experimental evidence that estrogen and/or progesterone may play important roles in ovarian

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carcinogenesis, including the control of cellular differentiation and growth, and apoptosis of ovarian cancer cells.[7] However, the nature of such roles is controversial.[4,5,7]

Apoptosis, or programmed cell death, is a vital physiologic process for the elimination of damaged or unwanted cells.[8,9] A hallmark of tumorigenesis is the outgrowth of a clonal population of cells that are insensitive to certain apoptotic signals. Various apoptotic stimuli signal through Bcl-2 family proteins in the core apoptotic machinery, and Bax is an essential factor in apoptosis initiation.[10]

In the present study, we used an established ovarian adenocarcinoma cell line, OC-117-VGH, which is negative for estrogen receptor (ER)α, ERβ and progesterone receptor (PR), to investigate the role of estrogen or progesterone in tumor cell growth using the CellTiter 96® AQ_{ueous} (CTAQ) Assay system (Promega US, Madison, WI, USA). We further evaluated regulators of tumor survival during estrogen or progesterone treatment. The selected candidate was the *Bcl-2* gene family, which has been widely considered to regulate cell death; thus, we also assessed changes in the expression of *Bcl-2* and *Bax*.

Methods

Cell line

An ovarian adenocarcinoma cell line, OC-117-VGH, was initiated from a clear cell adenocarcinoma that had been removed from a 56-year-old woman who underwent debulking surgery for surgicopathological stage IIIC ovarian cancer in 1997. The OC-117-VGH cell culture was late-passage (> 80) negative for ERα, ERβ and PR. We used the polymerase chain reaction (PCR) method[7] to prove the deficiencies in ERα, ERβ and PR in this cell line. The ERα (NM_000125) was amplified using the forward primer 5'–AGG–TGC–CCT–ACT–ACC–TGG–AGA–AC–3', and the reverse primer 5'–GGT–GGC–TGG–ACA–CAT–ATA–GTC–GTT–3' (amplicon length, 293 base pairs [bp]). The ERβ (NM_001437) was amplified using the forward primer 5'–GTT–TAG–TGG–TCC–ATC–GCC–AGT–TAT–3', and the reverse primer 5'–CTT–ACA–TCC–TTC–ACA–CGA–CCA–GAC–3' (amplicon length, 248 bp). The PR (NM_000926) was amplified using the forward primer 5'–GAT–TCA–GAA–GCC–AGC–CAG–AG–3', and the reverse primer 5'–TGC–CTC–TCG–CCT–AGT–TGA–TT–3' (amplicon length, 533 bp).

Human ovarian cancer cells (SKOV-3; American Type Culture Collection, Manassas, VA, USA),[11,12] which are positive for ERα but negative for ERβ and PR, were used as control.

Cell culture

OC-117-VGH cells were grown in a culture medium containing a 1:1 mixture of Dulbecco's modified Eagle's essential medium (Gibco BRL, Grand Island, NY, USA) and nutrient mix F-12 medium (Invitrogen Corp, Carlsbad, CA, USA), supplemented with 10% heat-inactivated fetal bovine serum (FBS; HyClone, Logan, UT, USA), penicillin 100 U/mL, streptomycin 100 μg/mL, and amphotericin B 2.5 μg/mL. Cells were incubated at 37°C with 5% CO_2, and at more than 95% humidity. SKOV-3 cells were maintained in culture at 37°C/5% CO_2 using Iscove's modified Dulbecco's medium (HyClone) supplemented with glutamine and 10% heat-inactivated FBS (HyClone). Media were changed twice a week, and cells were subcultured weekly by detachment with 2.5 mg/mL trypsin/ethylenediamine tetra-acetic acid 0.02% in Dulbecco's phosphate-buffered saline (PBS) solution (Gibco BRL). Cells were maintained and subcultured every 2–4 weeks and split at a 1:2 to 1:4 ratio prepared for subsequent studies.

Estrogen and progesterone treatment

Aliquots (100 μL) containing 1×10^6 cells/mL were dispensed into a 96-well plate (Corning Inc, Big Flats, NY, USA). Triplicate cultures for each test were incubated for 24 hours at 37°C, then 100 μL of different concentrations of estrogen and progesterone were added. Estrogen and progesterone were added separately to final concentrations of 0.01–1.0 μM and 0.1–10.0 μM, respectively, prepared in a serum-free medium. A serum-free culture medium was used for the control. Cells were treated with estrogen 0.01–1.0 μM and progesterone 0.1–10.0 μM for 16, 24, 48 and 72 hours at 37°C, with 5% CO_2 and more than 95% humidity.

Cell survival assay

Cell proliferation assays were examined using the CellTiter 96® AQ_{ueous} Assay system.[13] At the last 4-hour segment of estrogen and progesterone treatment, we added 20 mL per well of CTAQ. At the end, we read the plate with a microplate reader (Multiscan®, Labsystems, Helsinki, Finland) at an optical density (OD) of 490 nm.

Results were expressed as percent of cell survival after estrogen and progesterone treatment in culture media versus controls. Estrogen- and progesterone-treated survival rates at each concentration were calculated as follows:

$$\text{Survival rate relative to control (\%)} = \frac{\text{Absorbance of treated wells} - \text{mean absorbance of background}}{\text{Absorbance of control wells} - \text{mean absorbance of background}} \times 100\%$$

ELISA detection

Bcl-2 and *Bax* expression were determined by an enzyme-linked immunosorbent assay (ELISA) of the estrogen- and progesterone-treated cells. After 24, 48 and 72 hours of treatment, cells were washed with PBS and fixed in 75% ethanol for 10 minutes. This was followed by incubation with acetic acid 0.03% in PBS for 30 minutes. The cells were then incubated with rabbit anti-Bcl-2 or Bax primary antibodies (DakoCytomation California Inc, Carpenteria, CA, USA; 1:200 dilution) for 60 minutes at 37°C. After washing, the cells were then incubated with goat anti-rabbit alkaline phosphatase-labeled secondary antibodies (DakoCytomation California Inc; 1:500 dilution) for another 60 minutes at 37°C. Finally, the cells were incubated with 7 mM of p-nitrophenyl phosphate (Sigma-Aldrich Corp, St Louis, MO, USA) in a sodium bicarbonate buffer 0.01 M, pH 10.0, for 30 minutes, and then read using Multiscan® (Labsystems) at OD 410 nm.

The expression of *Bcl-2* and *Bax* at each condition was calculated as follows:

$$\text{Percentage relative to control (\%)} = \frac{\text{Absorbance of treated wells} - \text{mean absorbance of background}}{\text{Absorbance of control wells} - \text{mean absorbance of background}} \times 100\%$$

RNA purification and checking of the Bcl-2 and Bax-α genes using RT-PCR

We used the TRIzol® kit (Gibco BRL) to purify the RNA of ovarian cancer cells. We added 1 mL of TRIzol® solution to the drug-treated ovarian cancer cells, after centrifugation at 12,000*g* at 4°C for 30 minutes, collected the upper layer, and added isopropenol, after another centrifugation at 12,000*g* at 4°C for 30 minutes, and then washed the RNA twice with 70% alcohol.

We used the SuperScript® First-Strand Synthesis System (Gibco BRL) reverse transcriptase-polymerase chain reaction (RT-PCR) of RNA to cDNA, following the manufacturer's protocol, by incubating 1 μg of total RNA with oligo-d(T)12-18 (Invitrogen, Carlsbad, CA, USA) as the initiation primer in a final reaction volume of 20 μL, as described elsewhere.[14–16] The primers used for detecting genes by the RT-PCR method were as follows: the *Bcl-2* gene was amplified using the forward primer 5'–GGT–GCC–ATT–ATT–ATG–CAG–CCA–G–3' (GenBank® accession number M14745; nucleotides 722–743), and the reverse primer 5'–CCT–ATC–TGG–GCC–ACA–AGT–GAA–G–3' (GenBank® accession number M14745; nucleotides 875–899) (amplicon length, 178 bp).[17] The *Bax-α* gene was amplified using the forward primer 5'–ATG–GAC–GGG–TCC–GGG–GAG–CAG–C–3' (GenBank® accession number L22473; nucleotides 1–22), and the reverse primer 5'–CGA–GTC–GAA–GAA–CCA–CCT–GCG–T–3' (GenBank® accession number L22473; nucleotides 149–170) (amplicon length, 170 bp).[18] Expression of the *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) housekeeping gene was used to determine the cDNA yield and to check for possible contamination with genomic DNA. For this purpose, an intron-sparing *GAPDH*-specific primer (M17851) was designed: *GAPHD* forward primer, 5'–ATG–TTC–GTC–ATG–GGT–GTG–AAC–CA–3' (nucleotides 421–443), and *GAPDH* reverse primer, 5'–TGG–CAG–GTT–TTT–CTA–GAC–GGC–AG–3' (nucleotides 771–793) (amplicon length, 373 bp).[19]

Southern blotting

Southern blot hybridization was used to identify the PCR-amplified cDNA fragment. The PCR-amplified products were subjected to electrophoresis in 2% agarose gels and transferred onto nylon membranes. The DNA, linked to the nylon membrane by exposure to ultraviolet light (254 nm) at 1.5 J/cm^2, was prehybridized for 4 hours at room temperature in 6 M saline sodium citrate. cDNA probes 1–2 ng (above) were labeled with digoxigenin. Probe cocktails labeled with digoxigenin were used in the non-radioactive *in situ* hybridization method.[20]

Statistical analysis

Statistical analysis of the data was performed with Statistica version 5.1 (StatSoft Holdings Inc, Tulsa, OK, USA) and SPSS version 12.0 (SPSS Inc, Chicago, IL, USA). Every experiment was repeated 6 times.

Experimental data were presented as mean ± standard deviation, using Dunnet's test. Statistical analysis was performed by repeated analysis of variance (ANOVA), with a *post hoc* test for between-group analysis. A p value of less than 0.05 was considered statistically significant.

Results

Before we evaluated the effect of estrogen and progesterone on the survival of the OC-117-VGH ovarian adenocarcinoma cell line, we used PCR to identify the absence of functional ERα, ERβ and PR in the cell line (data not shown). Then, we evaluated the survival rate of the cell line treated with different estrogen concentrations (0.01–1.0 μM). Paradoxically, survival of cancer cells in this study was significantly impaired with estrogen treatment. Even at the low concentration of 0.01 μM, only 63.6 ± 6.7% of cancer cells survived; this impaired survival seemed to depend on estrogen concentration ($p = 0.02$). Furthermore, the longer the exposure of the OC-117-VGH cell line to estrogen, the greater the effect on cancer cell survival, especially at the highest estrogen concentration of 1.0 μM ($p = 0.04$); this suggested a time-dependent effect for estrogen on cancer cell survival at higher concentrations (Figure 1). By contrast, the various concentrations of estrogen did not significantly affect SKOV-3 cell growth (data not shown), which is in agreement with a previous report.[12] This SKOV-3 cell growth study indirectly suggested that estrogen did not cause cytotoxicity at any concentration.

We replaced estrogen with progesterone to evaluate the effect of progesterone on ovarian cancer cells. As predicted, progesterone showed similar but more significant effects than estrogen. We found that the survival of ovarian cancer cells was significantly impaired during progesterone treatment, in both a dose-dependent ($p = 0.001$) and exposure time-dependent manner ($p = 0.04$; Figure 2), thus suggesting that progesterone may be a protective factor against ovarian cancers. A similar phenomenon was also noted in the SKOV-3 cell line (data not shown), which is in agreement with a previous report.[11]

To further dissect the mechanism of estrogen or progesterone in the survival of OC-117-VGH cells, we used an ELISA assay and RT-PCR to check levels of the anti-apoptotic Bcl-2 protein and Bcl-2 mRNA expression. In agreement with the cell survival study, both protein and mRNA levels of Bcl-2 showed downregulation during estrogen treatment (66.2%, 58.3% and 55.1% of mean values at the 24-, 48- and 72-hour assessments, respectively; $p = 0.04$). This effect was more dramatic in the OC-117-VGH cells treated with progesterone (74.7%, 58.1% and 40.6% of mean values; $p = 0.01$). The downregulation of Bcl-2 was more significant with progesterone, because we could clearly determine the time-dependent effect of progesterone on Bcl-2 expression (Figure 3).

Since the balance between anti-apoptotic and pro-apoptotic signals plays a crucial role in cell survival, we further evaluated expression of the pro-apoptotic marker Bax on OC-117-VGH cells during estrogen and progesterone treatment, using both ELISA and

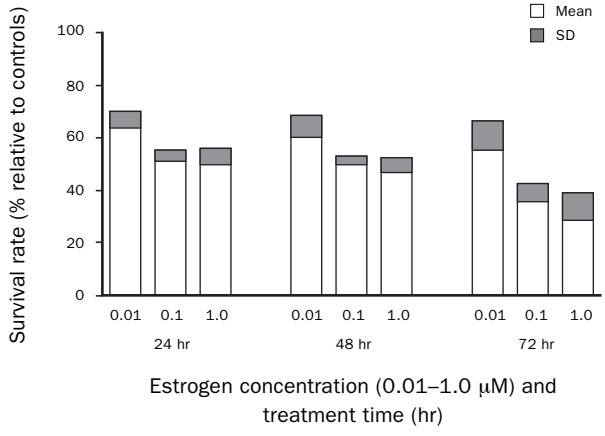

Figure 1. Survival rate of OC-117-VGH ovarian cancer cells treated with estrogen ($n = 6$). Concentrations of estrogen in the culture medium ranged from 0.01–1.0 μM. Impaired survival of the ovarian cancer cell line was estrogen-dose-dependent and estrogen-exposure time-dependent. SD = standard deviation.

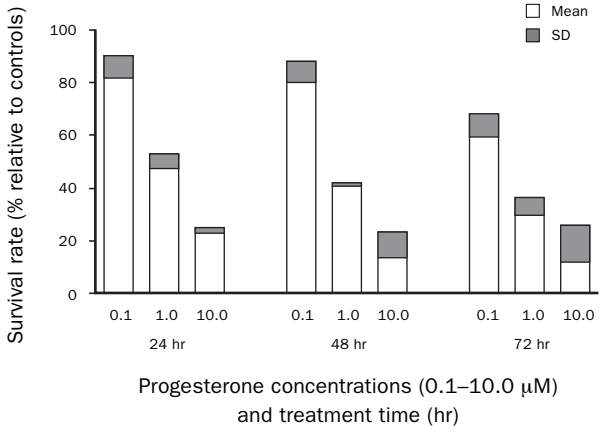

Figure 2. Survival rate of OC-117-VGH ovarian cancer cells treated with progesterone ($n = 6$). Concentrations of progesterone in the culture medium ranged from 0.1–10.0 μM. Impaired survival of the ovarian cancer cell line was progesterone-dose-dependent and progesterone-exposure time-dependent. SD = standard deviation.

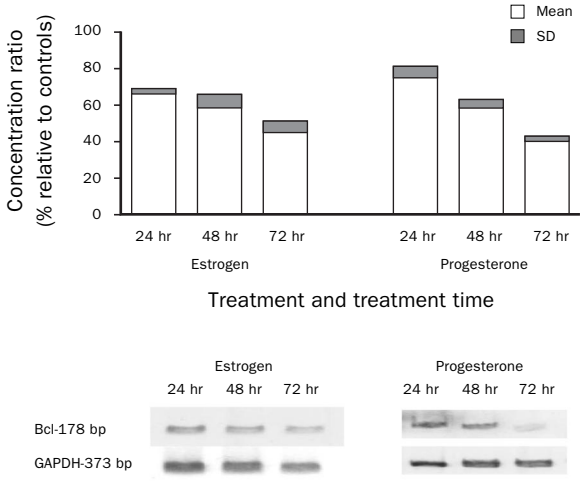

Figure 3. *Bcl-2* expression in OC-117-VGH ovarian cancer cells treated with estrogen or progesterone (n = 6). The estrogen concentration was 0.1 μM, whereas that of progesterone was 1.0 μM. Downregulation of *Bcl-2* expression was noted with estrogen and progesterone treatment. Southern blot hybridization showed the consistent finding of time-dependent decreased dosimetry density. bp = base pairs; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; SD = standard deviation.

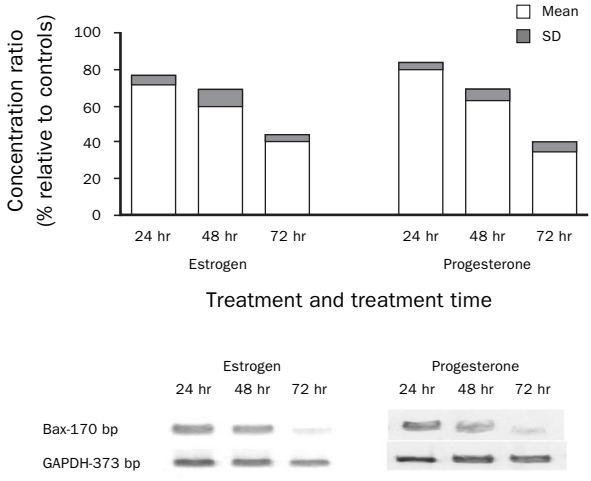

Figure 4. *Bax* expression in OC-117-VGH ovarian cancer cells treated with estrogen or progesterone (n = 6). The estrogen concentration was 0.1 μM, whereas that of progesterone was 1.0 μM. Downregulation of *Bax* expression was noted with estrogen and progesterone treatment. Southern blot hybridization showed the consistent finding of time-dependent decreased dosimetry density. bp = base pairs; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; SD = standard deviation.

RT-PCR. Paradoxically, both Bax protein and mRNA expression in OC-117-VGH cells were downregulated during estrogen treatment (72.4%, 60.2% and 39.1% of mean values at 24-, 48- and 72-hour assessments, respectively; $p = 0.01$). This phenomenon was also observed with OC-117-VGH cells treated with progesterone (79.3%, 64.6%, and 35.4% of mean values, respectively; $p = 0.001$; Figure 4).

Taken together, these results show that the OC-117-VGH cell line was sensitive to both estrogen and progesterone inhibition mediated through down-regulation of the anti- and pro-apoptotic genes *Bcl-2* and *Bax*. Although *Bax* was downregulated, it was the net effect of *Bcl-2* downregulation that impaired OC-117-VGH survival.

Discussion

The common epithelial ovarian tumors appear to arise from the ovarian surface epithelium, which is a simple squamous-to-cuboidal mesothelium covering the ovary.[21] However, the exact mechanism of ovarian tumorigenesis is not well known, although repeated ovulation contributes to neoplastic transformation of the ovarian surface epithelium, indicating that the process of healing ruptured ovarian surface epithelium may contribute to the disease.[22] Therefore, it has been suggested that endocrine and autocrine factors may influence the occurrence of ovarian tumors in women.[4] The WHI study suggested the probability of an increased risk of ovarian cancer associated with long-term use of hormone replacement therapy (HRT) with estrogen and progesterone.[6] However, this disagreed with other reports of an unchanged[23,24] or reduced risk of ovarian cancer.[25,26] Intriguingly, the Heart and Estrogen/progestin Replacement Study (HERS) II, a randomized trial of postmenopausal women, recently reported no difference in the incidence of ovarian cancer between an HRT and placebo group.[27]

Conflicting results from various studies and clinical trials may come from differences in methodology and the existence of uncontrolled confounding factors. Accumulation of evidence has long suggested estrogens as causative factors in ovarian cancer.[21,28–30] For instance, ovarian tissue estrogen levels are at least 100-fold higher than circulating levels, and levels are even higher in the follicular fluid of ovulatory follicles. Syed et al[31] also reported that both estradiol-17β and estrone stimulate growth of normal and malignant ovarian surface epithelium cells mediated through ERs. Importantly, estradiol-17β and estrone are of equal potency in stimulating growth in ovarian surface epithelium cells, although it is well known that estrone is a much less potent estrogen than estradiol-17β.

In addition, the mitogenic effects of estrogens on ovarian surface epithelium cells are mediated through activation of the interleukin-6/signal transducer and activator of transcription-3 (STAT-3) signaling pathway, and ovarian cancer cells express high levels of constitutively activated STAT-3, a known transforming cellular molecule.[32]

The mechanism of estrogen action is associated with upregulation of the *Bcl-2* gene at mRNA and protein levels, and this suggests a role for estrogen in ovarian tumorigenesis by preventing apoptosis in tumorigenic ovarian surface epithelium cells.[21] However, while the use of estrogen-based oral contraceptives (high-dosage estrogen) is known to reduce the risk of ovarian cancer, the ERα-positive cell line SKOV-3 fails to respond to estrogen stimulation,[12] as noted in this study. Furthermore, ERβ is reported to function as an important regulator of proliferation and motility of ovarian cancer and to have a pro-apoptotic role.[33] The loss of ERβ expression may thus be an important event leading to the development of ovarian cancer.[34] Lau et al[35] demonstrated expression of ERα, ERβ and PR mRNA in primary cultures of normal ovarian surface epithelium cells. A moderate reduction in ERα mRNA expression, accompanied by marked downregulation of PR expression, was noted in ovarian cancer cell lines when compared with ovarian surface epithelium cells.[35]

However, questions pertaining to the effects of estrogen on ovarian carcinogenesis remain unanswered.[22] Our study agreed with data from the above studies,[33,34] showing ERβ loss to be important in ovarian carcinogenesis, since the OC-117-VGH cell line was devoid of ERβ function. Interestingly, estrogen treatment in this cell line had a negative effect on tumor survival, which disagrees with the concept that estrogens are implicated as causative factors in ovarian carcinogenesis,[21,28–30] but seems to support the study of Sapi et al,[36] who showed that estrogen may induce apoptosis and thus impair cell survival; indeed, these investigators demonstrated estrogen-induced upregulation of Fasl, an apoptotic protein ligand, in ovary.[36] Sex hormones may also directly affect cell function and growth without binding to their relevant hormone receptors; i.e. these hormones can escape the classical pathway.[4,37,38] Overall, in this study, the effect of estrogen on OC-117-VGH survival and/or growth was not mediated through ERs, since both ERα and ERβ are defective in this cell line.

Progesterone, or the cellular response to progesterone, appears to offer protection against ovarian carcinogenesis.[11,22,29] The SKOV-3 cell line,[11,12] which is highly invasive, tumor-forming, and PR-negative, was used as an experimental paradigm to show that tumorigenesis is suppressed by progesterone in immunocompromised nude mice inoculated intraperitoneally with SKOV-3 cells.[11] Of significance, loss of heterozygosity at 11q23.3–24.3, which harbors the PR gene locus, is commonly observed in ovarian cancer specimens, and this genetic alteration is associated with poor prognosis.[39–41] Epidemiologic data provide additional support that progesterone or the response of ovarian surface epithelium cells to steroids affords a protective role against ovarian cancer development or progression.[24] An increase in ovarian cancer incidence was observed among women with progesterone deficiency,[42] and the protective effect of pregnancy may be attributable to exposure of the ovarian surface epithelium to high levels of progesterone during pregnancy.[43,44]

Furthermore, women with a history of twin pregnancies exhibit a lower risk of ovarian cancer, possibly due to the higher levels of progesterone found in the maternal circulation during twin rather than singleton pregnancies.[43,45] Indeed, it appears that progesterone protects against the development of common epithelial ovarian cancer.[29] In this study, use of the PR-negative cell line OC-117-VGH showed that progesterone may play a protective role in epithelial ovarian cancer, since progesterone successfully inhibited cell growth and reduced survival of the cell line; these findings were in complete agreement with previous studies.[11,22,42–45] However, progesterone activity may also be mediated through a non-genomic effect or non-PR-binding effect. Despite promising results from the abovementioned studies, progestogens have been of limited value in the treatment of ovarian cancer, even though they have sometimes been used in patients with advanced chemorefractory illness.[46,47] Our observations provide a conceptual basis for the prophylactic and therapeutic use of progesterone in individuals at high risk of ovarian carcinoma, and in those diagnosed with early-stage disease. We further hypothesize that high progesterone dosages may be appropriate to achieve beneficial outcomes, since adverse effects with progesterone in its natural form are expected to be low.[47]

To clarify regulators of tumor survival during estrogen and progesterone treatment, we further studied the *Bcl-2* gene family, members of which are generally considered to be regulators of cell death,[48,49] although the prognostic significance of *Bcl-2* and/or *Bax* remains controversial.[50–52] Among pro- and anti-apoptotic genes in the *Bcl-2* family, *Bax* and *Bcl-2* genes are dominant regulators of apoptosis. The ratio

of *Bcl-2* to *Bax* is critical in determining susceptibility to apoptosis,[49] and steroid hormones may regulate pro- or anti-apoptotic genes in ovarian cancer cells.[21,29,53] In this study, estrogen (0.01–1.0 μM) and progesterone (0.1–10.0 μM) decreased both Bax and Bcl-2 protein, suggesting that a decrease in Bcl-2 protein plays a more important role that is unfavorable to cell survival. The net effect of a decreased relative ratio of *Bcl-2* to *Bax* decreased survival and/or growth in the OC-117-VGH cells.

Regarding study limitations, as the OC-117-VGH cell line is deficient in ERs and PRs, the effects of estrogen or progesterone on cell survival may not be mediated through classical pathways (i.e. estrogen/ER, and progesterone/PR); however, we did not examine non-classical pathways (i.e. non-genomic effects, or non-ER- or non-PR-binding effects) to prove our observation that both estrogen and progesterone decreased survival and/or growth in OC-117-VGH cells. We did not quantify the induction of apoptosis; indeed, DNA fragmentation was measured using a cell-death detection ELISA (Roche Molecular Biochemicals, Mannheim, Germany) as previously reported.[21] We also failed to detect the typical morphologic characteristics of apoptosis, such as compaction and margination of chromosomes, nuclear fragments, and the formation of apoptotic bodies. Therefore, it was difficult to claim that the decreased survival and/or growth may have been due to factors other than cytotoxicity, although based on the above SKOV-3 study and previous reports,[7,12,21,32,33] such cytotoxicity was unlikely. The decreased human cancer cell population *in vitro*, as observed in this study, reflects a change in the balance of cellular growth events and could involve arrested cell proliferation, enhanced cell death, or both;[34] however, we did not classify which one was predominant. Lastly, to detect changes in *Bcl-2* and *Bax*, we used a very sensitive RT-PCR method, which might amplify small changes that have no clinical significance.

In conclusion, the present study indicated that both estrogen and progesterone impaired tumor survival in the OC-117-VGH ovarian cancer cell line. Although anti-apoptotic Bcl-2 and pro-apoptotic Bax proteins were downregulated by estrogen and progesterone, decreased Bcl-2 played the most important role in contributing to the net effect of reduced ovarian cancer cell survival. Our results also confirm that estrogen and progesterone can be used as chemopreventive agents against ovarian cancer, especially against ER-negative and PR-negative disease.

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