Re-expression of estrogen receptor β inhibits the proliferation and migration of ovarian clear cell adenocarcinoma cells

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Abstract. Ovarian clear cell adenocarcinoma (OCCA) is an aggressive ovarian malignancy with a poor prognosis. The role of estrogen receptor β (ERβ) in the development of OCCA remains to be clarified. To investigate the action of ERβ in the proliferation and invasion of OCCA cells, the ES-2 cell line was stably transfected with full-length human ERβ cDNA, and clones were screened and identified using RT-PCR and Western blot assay. ERβ stable transfectants, referred to as ESβ1 and ESβ2 cells, were compared with mock transfected ESVE and parental ES-2 cells with respect to their growth, motility and ability to activate target genes. ESβ1 and ESβ2 cells expressed ERβ mRNA and protein, whereas ES-2 and ESVE cells were ERβ negative. ERβ transfectants exhibited distinct characteristics from ES-2 and ESVE cells including proliferative properties and the ability to express cyclin D1 in the presence of 17β-estradiol (E2). ERβ inhibited ES-2 cell proliferation, which was determined using the MTT assay, BrdU labeling method and by the down-regulation of cyclin D1 gene expression. Moreover, exogenous ERβ expression resulted in a significant inhibition of ES-2 cell motility in an in vitro invasion assay. ERβ reduced the expression of MMP2 mRNA and the activity of MMP2 enzymatic activity in a ligand-dependent manner. In summary, ERβ may inhibit the proliferation and invasion of ES-2 cells and may be an important regulator in OCCA carcinogenesis.

Introduction

Ovarian clear cell adenocarcinoma (OCCA) has been defined as a distinct histopathological subtype of epithelial ovarian cancer (EOC) by the World Health Organization (WHO) since 1973. OCCA is thought to include 5-10% of all ovarian carcinoma in Western countries and an even higher percentage in Japan. In an investigation of 28,082 women with EOC, Chan et al reported that 1,411 (5%) were diagnosed with OCCA, and women with OCCA were younger, more likely to be Asian, and their prognosis was typically worse than serous cancers (1). The clinical management of advanced EOC included maximal cytoreduction and a regimen of platinum-based chemotherapy (2). Pectasides et al reviewed 54 articles concerning OCCA and supported the hypothesis that OCCA was a biologically distinct entity. OCCA patients were found to have a high incidence of stage I disease because it frequently presented with a large pelvic mass. Recurrences of OCCA were more frequent than other types of EOC (3). Patients with OCCA responded poorly to conventional paclitaxel and platinum-based chemotherapy as well as platinum-free chemotherapy.

According to several authors, endometriosis was associated with OCCA in 20-50% of patients, which is more prevalent than serous adenocarcinoma. Endometriosis-associated early ovarian clear cell adenocarcinoma demonstrated lower tumor proliferation than adenocarcinoma without endometriosis, which indicates differences in their biological characteristics. Mutual interactions mediated through cytokines, such as TGF-β1, might occur between endometriosis and OCCA, while endometriosis-derived factors might negatively regulate tumor proliferation (4-6). Akahane et al proposed that a disappearance of steroid hormone dependency might be involved in the malignant transformation of endometriosis into OCCA (7).

The proliferative effect of estrogen has been observed in different estrogen receptor-positive ovarian cancer cells. Syed et al reported that normal and malignant OSE cells exhibited estrogen receptor (ER) mediated growth stimulatory responses to 17β-estradiol and estrone. The mitogenic effect of estrogens on OSE cells was mediated through activation of the IL-6/STAT-3 signaling pathway (8,9). ERβ might play a protective role against ERα mitogenic activity. Estrogen-induced gene expression through ERβ may also provide a new therapeutic target (10). These studies introduced the hypothesis that molecular activation of ERβ or inducing ERβ re-expression in neoplastic cells may help prevent tumor proliferation or invasion. To help define the role of ERβ in an ovarian clear cell adenocarcinoma cell line, we introduced full-length human ERβ cDNA into ES-2 cells. In this study, we investigated growth rates and cyclic changes in the proliferative activity of ES-2 cells and motility mediated by exogenous ERβ in the ovarian clear cell line. To better determine the molecular
Table I. Primer sequences for RT-PCR amplification.

<table>
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<th>Target</th>
<th>Base pair</th>
<th>Annealing temperature (˚C)</th>
<th>Sequences 5’-3’</th>
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</thead>
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<td>ERβ</td>
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<td>53</td>
<td>TGCTTTGGTITGGGTAGTGC</td>
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<tr>
<td>GAPDH</td>
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<td>60</td>
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<td>Cyclin D1</td>
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<tr>
<td>MMP-2</td>
<td>398</td>
<td>55</td>
<td>CCATGCCAGTGACCTCCTCCTAC</td>
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</tbody>
</table>

mechanism underlying the effects of ERβ modulation, we examined target genes involved in proliferation and invasion of ES-2 cells regulated by constitutive ERβ.

Materials and methods

Cell lines and culture conditions. Human OCCA ES-2 cells were purchased from the American Type Culture Collection (ATCC, Rockville, USA). Human fibroblast NIH-3T3 cells were gifts from the Breast Cancer Department of Fudan University, China (Shao Zhimin, MD). Cells were cultured in McCoy-5a (Gibco, USA) supplemented with 10% fetal bovine serum (FBS) in a humidified atmosphere with the presence of 5% CO₂ at 37˚C. Prior to experiments, cells were washed with phosphate-buffered saline (PBS) and maintained in phenol-red free Mc'Coy-5a (Gibco, USA) containing 5% dextran-coated charcoal (DCC)-stripped FBS (Biological Industries, Israel) and were treated with 10⁻⁸ M 17β-estradiol (E₂) (Sigma, USA).

Plasmid and stable transfection. The expression plasmid pBK-RSV and the ERβ cDNA containing plasmid pBK-RSV-ERβ were obtained from Uniformed Services University of the Health Sciences, Bethesda, USA (Paul H. Driggers, MD) (34). ES-2 cells were seeded into 24-well plates at a density of 5x10⁴ per well in Mc'Coy-5a supplemented with 10% FBS and grown to 90-95% confluence. Cells were co-cultured with pBK-RSV-ERβ DNA and Lipofectamine 2000 (Invitrogen, USA) as recommended by the manufacturer. Twenty-four hours after transfection, cells were trypsinized and detached in a 100 mm petri plate at a 1:10 ratio and cultured in the presence of Geneticin (G418, InvivoGen, USA, 800 µg/ml media). The individual G418-resistant colonies were picked and maintained after two weeks. Expression of ERβ was verified by RT-PCR and a Western blot assay. A vector control cell line was established by transfecting an empty expression plasmid.

RNA isolation and reverse transcription PCR. Total RNA was isolated using TRIzol (Invitrogen, USA) as recommended by the manufacturer and precipitated with 75% ethanol using standard procedures. Total RNA (0.5 µg) from each sample was subjected to reverse transcription using the M-MuLV Reverse Transcriptase RNase (Fermantas, Canada) and oligo dT primers (Fermantas) in a 20-µl reaction volume. The PCR reactions were performed using 1 µl of the RT mixture in a final volume of 25 µl with Taq DNA polymerase (Fermantas). To detect ERβ, PCR-amplification involved a denaturation step at 94˚C for 5 min. Cycles including 45 sec melting at 94˚C, 30 sec annealing at 53˚C, and 45 sec of extension at 72˚C were performed 30 times followed by a final elongation at 72˚C for 10 min. All PCR primer sequences and reaction conditions are indicated in Table I. The amplified products were separated by electrophoresis on 1.5% agarose gel and identified by ethidium bromide staining under UV illumination.

Western blot analysis. Cells were grown to 90-95% confluence on 50-mm petri plates, placed on ice and washed with PBS, lysed in 100 µl of a protein extraction buffer (10 mmol/l Tris pH 7.4, 1.5 mmol/l EDTA, 130 mmol/l NaCl, 1% Triton, 10 mmol/l NaF, 10 mmol/l NaPi, 10 mmol/l NaPi, 10 mmol/l NaPPI, 5 µg/ml aprotinin, 5 µg/ml leupeptin, 100 µg/ml PMSF, and 0.25 mmol/l NaVO₄) containing a protease inhibitor cocktail (Roche) and incubated with intermittent mixing for 30 min. Whole cell extracts were collected and debris was removed after centrifugation. Protein concentrations were measured using a protein microassay kit (Shennengbocai, Shanghai, China). Aliquots of 80 µg total protein of were separated through 12% SDS-polyacrylamine electrophoresis gels (Bio-Rad Mini-Gel Box Electrottransfer) and transferred to a PVDF filter (Pall-Gelman, USA). The PVDF filter was developed with primary antibodies (anti-ERβ, Upstate, USA, 1:2000; cyclin D1, Neomarkers, USA, 1:500; β-actin, Abcam, UK, 1:5000) overnight at 4˚C, blocked in 5% non-fat dry milk (PBS+0.1% Tween-20), and incubated with a horseradish peroxidase (HRP)-conjugated anti-rabbit/mouse antibody (1:20000, Qikang, Beijing, China) for 1 h at room temperature (RT). Proteins were detected using the ECL kit (Pierce Biochemicals, Rockford, IL) and chemiluminescent detection system (Alpha Innotech).

Cell proliferation assay. Two thousand cells were plated in 200-µl media per well in a 96-well plate and incubated (37˚C, 5% CO₂) overnight to allow the cells to attach to the wells. Eight wells were left empty for blank controls. Then cells were washed in PBS and cultured in phenol red-free Mc'Coy-5a with 5% DCC-FBS in the presence of 10⁻⁸ M 17β-estradiol for 1-7 days. The medium was changed every 3 days. A solution of 5 mg/ml thiazolyl blue tetrazolium bromide (MTT) dissolved
in PBS was made and filter sterilised through a 0.2-µM filter. MTT solution (20 µl) was added to each well. The plate was incubated at 37°C for 4 h. Media were removed with needle and syringe. Dimethyl sulfoxide (150 µl) (DMSO) was added to each well and the plate was placed on a shaking table (150 rpm for 5 min). Optical absorbance (OA value) at wavelength 492 nm and subtract background at 560 nm was measured.

BrdU labeling method. Cells were incubated with BrdU (10 µmol/l, Sigma) for 1 h at 37°C and fixed with 4% paraformaldehyde for 20 min at room temperature. Following fixation, cells were washed in 0.1 M PBS (pH 7.4) (3x5 min) and incubated in HCl (2 N) for 1 h at 37°C. Immediately after the cells were acid washed, borate buffer (0.1 M) was added for 30 min at room temperature. Cells were then washed in 0.1 M PBS (3x5 min) and PBS with 1% Triton X-100 for 2 min at room temperature. Prior to incubation with anti-BrdU (Sigma, 1:200), cells were blocked with 10% BSA for 1 h at room temperature. Following the incubation, the cells were washed in 0.1 M PBS (pH 7.4) with 0.1% Tween-20 (3x5 min) and were treated with the HRP conjugated secondary antibody to visualize the anti-BrdU-labeled cells with diaminobenzidine (DAB). To measure the labeling index (LI), the following formula was applied: BrdU LI = BrdU labeled cells/total cells counted x 100%.

Gelatin zymography. The running gel (10%) was prepared with 0.2% gelatin (2 mg/ml). The samples were applied (10 µg protein in total medium), and the gel was run with a 1X Tris-Glycine SDS buffer according to the standard running conditions (100 V, constant voltage). Afterwards, the gels were incubated in a zymogram renaturing buffer (2.5% Triton X-100) with gentle agitation for 30 min at room temperature, washed in a Tris-cl buffer (50 mmol/l), and replaced with a zymogram developing buffer. The gel was equilibrated for 30 min at room temperature with gentle agitation and incubated with fresh zymogram developing buffer at 37°C for 42 h. Gels were stained with 0.05% (w/v) Coomassie blue for 24 h at room temperature, destained for 8 h in a destaining solution (methanol:aceticacid:water, 10:10:80). Areas of protease activity appeared as clear bands against a dark blue background where the protease had digested the substrate.

Transwell migration assays. Cells (1x10⁵) were placed in the upper chamber of 8 micron transwells (Costar, USA) on 100 µl of diluted Matrigel (BD Biosciences, Bedford, MA) with NIH 3T3 derived supernatants in the lower chamber and allowed to migrate for 24, 48, or 72 h at 37°C. After the appropriate time, the filter side of the upper chamber was cleaned with a cotton swab, and the filter was stained according to the standard H&E staining procedure. The filter was gently cut from the chamber, and cells that had migrated through the filter pores were counted. Three identical replicates were performed for each migration condition.

Densitometric and statistical analysis. The intensities of mRNA and protein bands of RT-PCR and Western blot assays were determined and calculated in relation to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA or β-actin protein levels. Statistical differences were calculated using the χ² or t-test with SPSS 12.0 software.

Results

Stable transfection of ES-2 cells with ERβ. To assess the contribution of ERβ in ovarian clear cell adenocarcinoma and its underlying molecular mechanisms, stable ERβ transfectants were established. Following antibiotic selection, several clones were identified and screened for ERβ expression using RT-PCR and Western blot analysis. ES-2 cells used in our experiments were ERβ-negative. Two clones, ESβ1 and ESβ2, with high levels of transfected ERβ expression were chosen for further studies. As a control, ES-2 cells were also transfected with an empty expression vector termed ESVE. RT-PCR products of ERβ mRNA were detected in transfected ESβ1 and ESβ2 cells but not in ES-2 and ESVE cells (Fig. 1A). Western blot analysis confirmed that ESβ1 and ESβ2 cells expressed ERβ, while parental ES-2 and mock transfected ESVE cells were ERβ negative (Fig. 1C). Continued expression of ERβ was routinely verified throughout the study. ERβ-positive mRNA and protein from human testis were used as a positive control.

Inhibition of ERβ stable transfectants growth by ERβ expression. We assessed the growth rate of ES-2, ESVE, ESβ1 and ESβ2 cells in vitro using an MTT assay. Initially, a dose-response analysis for E2 was conducted (concentration range: 10⁻⁸ to 10⁻¹⁰ M) (data not shown), and further analyses were performed at an E2 concentration of 10⁻⁸ M. Cells were maintained in steroid-depleted medium containing charcoal-stripped FBS and E2. After 24 h of E2 treatment, ESβ1 and ESβ2 cells began to demonstrate a reduced growth rate compared to ESVE and ES-2 cells. After 7 days, ESβ1 and ESβ2 cells showed growth retardation compared to non-transfected and mock-transfected cells (Fig. 2). When cultured in normal medium (i.e., McCoy-5a with 10% FBS for 7 days), ESβ1 and ESβ2 cells also presented growth retardation, although it was less apparent than with E2 treatment. All of the results showed statistical differences in tumor cell growth between ERβ-transfected cells and ERβ-negative cells.
Effects of ERβ on cell cycle progression and endogenous target genes. To study the effects of ERβ on the cell cycle, cells were plated and analyzed using BrdU assays. Fig. 3 shows the BrdU-labeled nucleus of non-transfected, mock-transfected, and ERβ-transfected cells. BrdU LIs of ES-2 and ESVE cells were 32.03±1.79 and 32.20±1.65%, respectively. The LI of ERβ-transfected ESβ1 cells was 12.03±0.81%, which was less than ES-2 and ESVE cells (P<0.01). No differences in BrdU LI were observed between ES-2 and ESVE cells (P>0.05).

We analyzed the cell cycle protein cyclin D1, which is associated with proliferative status and S-phase progression. After treatment of ES-2, ESVE, ESβ1, and ESβ2 cells with 10⁻⁸ M E₂ for 72 h, the expression of cyclin D1 mRNA and protein was monitored by RT-PCR and Western blot analysis, respectively. Visualization of RT-PCR products by ethidium bromide staining was performed to demonstrate the relative gene expression of cyclin D1 relative to GAPDH. Proteins were detected by Western blot analysis and semi-quantified in relation to β-actin levels. Each experiment was performed independently in triplicate. The expression of mRNA and protein of cyclin D1 was equal in ES-2 and ESVE cells. The cyclin D1 mRNA levels in ESβ1 and ESβ2 cells were 50.50±0.95 and 50.83±1.62%, respectively, lower than cyclin D1 mRNA levels in ESVE cells (P<0.01). The relative protein expression was also lower in ESβ1 and ESβ2 cells (41.00±2.88 and 42.47±1.50%, respectively, lower than the cyclin D1 protein level in ESVE cells, P<0.01), as shown in Fig. 4. These results were consistent with the BrdU-labeling analysis and approved the delayed S-phase entry of ERβ-transfected ES-2 cells.

ERβ exerted influence on the invasion of OCCA

ERβ inhibit motility of ES-2 cells. After incubation of cells with 10⁻⁸ M E₂ for 24, 48 or 72 h, the total number of ES-2, ESVE, and ESβ cells that migrated through the membrane was counted in a x200 field under a microscope. Data are reported as the average of experiments for each time setting.

After incubation for 24 h, the numbers of migrating ES-2, ESVE, and ESβ cells per x200 field were 68.00±7.00, 77.33±8.02, and 21.67±5.03, respectively; after 48 h, the numbers of migrating ES-2, ESVE, and ESβ cells were 101.67±7.47, 127.67±15.01, and 38.33±9.07, respectively; after 72 h, the numbers of migrating ES-2, ESVE, and ESβ cells were 333.00±25.53, 366.00±26.51, and 54.33±14.01, respectively. Fig. 5 showed that the migration of ERβ transfected cells was lower than parental ES-2 and mock-transfected cells after 24, 48, and 72 h of E2 treatment (P<0.01). There was no difference in migration between E2-pretreated ES-2 and ESVE cells for each time setting (P>0.05). These results

Figure 2. Effect of ERβ-transfection on proliferation of ES-2 cells in vitro. Growth rate was evaluated by MTT assay after 1, 3, 5 and 7 days of exposure to 10⁻⁸ M estradiol. ESβ1 and ESβ2 cells showed a significantly slower rise in cell numbers than ES-2 and ESVE cells at each selected time. Independent experiment was performed in triplicate. P<0.01.

Figure 3. Immunocytological analysis revealed homogeneously stained BrdU-positive nuclei of ES-2, ESVE, and β-transfected ESβ1 cells (H&E staining, x200). (A) BrdU-labeled ES-2 cells; (B) BrdU-labeled ESVE cells; (C) BrdU-labeled ESβ1 cells; (D) comparison of BrdU labeling index of ES-2, ESVE and ESβ1 cells. Each experiment was performed in triplicate. **P<0.01.
suggested that the expression of ERβ decreased the migration of ES-2 cells.

Inverse regulation of MMP-2 gene expression and activity by ERβ. After incubation with 10^{-8} M 17β-estradiol for 72 h, re-expression of ERβ caused a decrease in MMP-2 mRNA production in ESβ1 and ESβ2 cells compared to mock-transfected ESVE and parental ES-2 cells. The ratios of relative MMP-2 mRNA band densities of ESβ1 or ESβ2 vs. ESVE cells were 25.70±0.26% (P<0.01) and 27.23±0.75% (P<0.01), respectively (Fig. 6A).

Gelatin zymography also showed that MMP-2 activity in culture medium from ERβ re-expressing cells was far lower than ESVE and ES-2 cells. The active bands for MMP-2 (62 kDa) and proMMP-2 (64 kDa) are shown in Fig. 6C. Gelatinolytic activities of 62 kDa bands were subjected to densitometric analysis, which showed that the activities of MMP-2 were significantly lower in ESβ1 and ESβ2 cells (ESβ1 vs. ESVE, 49.80±1.35%; ESβ2 vs. ESVE, 50.77±1.66%, P<0.01). Therefore, MMP-2 mRNA production and activity appeared to be negatively regulated by ERβ. These results showed that ERβ suppressed MMP-2 mRNA expression of ES-2 cells in a ligand-dependent manner; furthermore, this suppression was reflected in the MMP-2 activity as determined by gelatin zymography, as shown in Fig. 6D.

Discussion

ERβ is a member of the superfamily of nuclear receptors first cloned in 1996 and is highly homologous with the classical functional estrogen receptor ERα (11,12). ERβ binds estrogens with an affinity similar to ERα and mediates estrogen-dependent expression of reporter genes containing estrogen response elements (ERES). ERα and ERβ can dimerize with each other. Dimerization may increase the complexity of transcription activation and suggests the existence of two previously unrecognized estrogen-signaling pathways, ERβ homodimers and ERα/ERβ heterodimers. These dimers may cooperate in the regulation of estrogen-
responsive gene expression in cell types in which they are co-expressed (13).

Some evidence has shown that ERβ may inhibit tumor development and was considered to be a negative dominant of ERα. Rutherford et al observed varying amounts of ERα and ERβ in normal ovaries, lower levels of ERβ expression in primary tumors of ovarian epithelial cancer, and only ERα in metastatic tumors. ERβ mRNA and protein levels decreased in patients with ovarian cancer and metastases (14). Other studies have shown that ERβ mRNA levels decrease or that the ERα/ERβ mRNA ratio increase during ovarian tumor progression (15-17). Other cancers, including breast, prostate and colon, have reduced expression of ERβ mRNA and/or protein (or an increased ERα/ERβ mRNA ratio) as well (18-23). The mechanisms involved in the decreased ERβ expression in tumors remains elusive. The ERα/ERβ ratio is a decisive parameter in orienting the transcriptional mechanism of a target gene in the presence of agonist and antagonist ligands (24). The expression of different gene populations may be affected by the dimer composition of two ER subtypes. The dominant negative properties of ERβ are mainly due to its AF-1 function.

This study focused on the activities of exogenous full-length ERβ cDNA in ES-2 cells (an OCCA cell line, in which ERα and ERβ expression were negative) and transcriptional modulation of target genes in the aggressive cancer cells. One of the most important issues of ovarian clear cell adenocarcinoma is tumor proliferation. Our study provided evidence that ERβ suppressed the growth of ovarian clear cell adenocarcinoma cells. Transfection of ERβ into ES-2 cells resulted in a significant inhibition of cellular growth in vitro and delayed transition into the S-phase of the cell cycle. Growth was further inhibited in an estrogen-dependent manner. Other studies support the idea that decreased ERβ expression could lead to breast and ovarian cancer development (15,25,26). Furthermore, ERβ expression in breast cancer cells appears to favor anti-estrogen treatment (24).

As we described, constitutive expression of ERβ significantly reduced the percentage of cells in the S-phase. To label S-phase cells, bromodeoxyuridine (BrdU), a thymidine analogue, can be incorporated into newly synthesized DNA in S-phase cells. As shown in Fig. 3, transfection of ERβ into ES-2 cells significantly decreased the BrdU labeling index in an estrogen-dependent manner. To assess the molecular mechanism of the G1-S transition of the cell cycle, we transfected ES-2 cells with ERβ and observed that cyclin D1 mRNA and protein expression was repressed. This result indicates that constitutive ERβ might reduce the expression of cell cycle genes, such as cyclin D1, and thus inhibit cell proliferation. ERβ has been shown to repress c-myc, cyclin D1, cyclin A, and Cdk2 and increase the expression of p21wafl/cip1 and p27kip1 in MCF-7 breast cancer cells (25,27-29). It also decreased the amount of cyclin E, cdc25A, and p45skp2 in T47D breast cancer cells (30). Furthermore, endogenous ERβ is one of the mediators of the antiproliferative action of estrogens in enhancing the synthesis of molecules, such as p21, that control the cell cycle in prostate cancer cells (31). All of these observations indicate that ERβ acts as a tumor suppressor.

Invasion is another important event in highly aggressive ovarian clear cell adenocarcinoma. In this study, we observed that constitutive expression of ERβ could decrease the migration of ES-2 cells over different time settings. Identification of the mechanism of ERβ modulation of tumor migration is of considerable interest. Due to its simplicity and sensitivity, gelatin zymography was used for matrix metalloproteinase measurement. MMP-9 and MMP-2 were secreted by cells. MMP-2 and not MMP-9 activity was suppressed on a gelatin zymogram with stable ERβ transfection. These results, along with decreased MMP-2 mRNA expression in ESβ cells, indicate that constitutive ERβ suppresses MMP-2 expression at the transcriptional level with a subsequent reduction in enzyme protein content. Wound healing-induced migration experiments were performed on ovarian cancer PEO14 cells and it was observed that ERβ-transfected cells had very slow migration ability (15). Decreased migration ability as a result of constitutive ERβ has also been described previously in breast and prostate cancer (26,32).

Our data provide strong support to the hypothesis that ERβ acts as a tumor suppressor in ovarian clear cell adenocarcinoma via its growth-blocking action. The present study demonstrated that stable transfection of an ERβ isoform into ES-2 cells inhibited the G1-S transition during the cell cycle and reduced cyclin D1 expression. Data from multiple experiments indicate that ERβ plays an important role in the proliferation of ES-2 cells. It would also be interesting to investigate whether exogenous ERβ expression in ES-2 cells leads to a difference in motility ability and an associated repression of MMP-2 activity. Our study might provide a more complete understanding of the function of ERβ and its signal transduction pathway in aggressive ovarian clear cell adenocarcinoma. Re-expression or molecular stimulation of ERβ may be a
potential therapeutic approach. Further studies are necessary to clarify the mechanism of gene modulation (such as methyla
tion of the gene promoter or another transcriptional control) of the ERβ signal transduction pathway in carcinogenesis.

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References