Estrogen Receptor β Inhibits Angiogenesis and Growth of T47D Breast Cancer Xenografts

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Abstract

Estrogens, which are stimulators of growth of both the normal breast and malignant breast, mediate their effects through two estrogen receptors (ER), namely ERα and ERβ. ERα mediates the proliferative effect of estrogen in breast cancer cells, whereas ERβ seems to be antiproliferative. We engineered ERα-positive T47D breast cancer cells to express ERβ in a Tet-Off–regulated manner. These cells were then injected orthotopically into severe combined immunodeficient mice, and the growth of the resulting tumors was compared with tumors resulting from injecting the parental T47D cells that do not express ERβ. The presence of ERβ resulted in a reduction in tumor growth. Comparison of the ERβ-expressing and non-ERβ–expressing tumors revealed that the expression of ERβ caused a reduction in the number of intratumoral blood vessels and a decrease in expression of the proangiogenic factors vascular endothelial growth factor (VEGF) and platelet-derived growth factor β (PDGFβ). In cell culture, with the Tet-Off–regulated ERβ-expressing cells, expression of ERβ decreased expression of VEGF and PDGFβ mRNA under normoxic as well as hypoxic conditions and reduced secreted VEGF and PDGFβ proteins in cell culture medium. Transient transfection assays with 1,026 bp VEGF and 1,006 bp PDGFβ promoter constructs revealed a repressive effect of ERβ at the promoter level of these genes. Taken together, these data show that introduction of ERβ into malignant cells inhibits their growth and prevents tumor expansion by inhibiting angiogenesis. (Cancer Res 2006; 66(23): 11207-13)

Introduction

Breast cancer is one of the leading forms of cancer in the Western world. It is well documented that the mitogenic actions of estradiol (E_2) are critical in the etiology and progression of human breast cancers (1). For this reason, patients with estrogen receptor α (ERα)–positive tumors are treated with tamoxifen, which blocks the action of ERα, or with aromatase inhibitors, which block the synthesis of E_2 (2–4). These treatments are effective but patients inevitably develop hormone-resistant, invasive tumors. Because ERβ is expressed in the normal breast, it is pertinent to ask whether this receptor might also be used as pharmacologic target in the treatment of breast cancer.

In breast cancer cells as well as in various mouse models, estrogens via ERα stimulate proliferation and inhibit apoptosis (5, 6), whereas ERβ opposes the proliferative effect of ERα *in vitro* (7, 8). Several studies have reported that during tumor development in the breast epithelium, the expression of ERα increases, whereas that of ERβ decreases (9, 10). For a tumor to grow, increased proliferation must be accompanied by increased blood supply and this is achieved by angiogenesis and increased blood microvessel density (11–13). Angiogenesis is an essential process in normal tissue growth and repair and is regulated by multiple factors, including supply of nutrients, vascular endothelial growth factor (VEGF), platelet-derived growth factor β (PDGFβ), basic fibroblast growth factor (bFGF), and oxygen tension. Estrogens are important stimulators of angiogenesis in health as well as disease. In the uterine endometrium, estrogens via ERα increase the transcription of VEGF (14, 15) and stimulate angiogenesis. Tamoxifen is antiangiogenic (16, 17).

The question addressed in the present study is whether an established breast cancer cell, which does not express ERβ, can be influenced to behave more like a normal cell upon reintroduction of ERβ. To answer this question, we engineered T47D breast cancer cells with a tetracycline-regulated ERβ expression vector. We show here that both proliferation of the cancer cell and its ability to grow as a solid tumor in severe combined immunodeficient (SCID) mice were reduced upon expression of ERβ.

Materials and Methods

Stable cell lines and cell cultures. T47D cells were grown in DMEM/Ham's F-12 (1:1) (Invitrogen) supplemented with 5% fetal bovine serum (FBS). T47D cells stably transfected with tetracycline-regulated ERβ expression plasmid were generated in two steps. The cells were first transfected with pTet-tTAk (Life Technologies) modified to contain puromycin resistance using Lipofectamine according to instructions of the manufacturer (Life Technologies). Selection was done with 0.5 μg/mL puromycin in the presence of 1 μg/mL tetracycline. A clone showing high levels of induction upon tetracycline withdrawal and low basal activity was selected by using the pUHC13-3 control plasmid (Life Technologies). The short form of ERβ encoding the COOH-terminal 485 amino acids [the reason why the long form of ERβ (530 amino acids) was not used was that it was lethal to the cells] was fused to the FLAG tag (ERβ 485) and cloned into pBI-EGFP (Clontech). This construct was then transfected into the previously described inducible cell clone together with a neomycin resistance plasmid, and selection was done with 500 μg/mL G418 (Calbiochem), 1% penicillin-streptomycin (Invitrogen), and 0.1% kanamycin (Sigma, St. Louis, MO). For experimental conditions, stripped medium was used, i.e., phenol red–free medium (Invitrogen) supplemented with dextran/charcoal–treated FBS. E_2 (Sigma) was dissolved in ethanol and 4OH-tamoxifen (Sigma) was dissolved in DMSO.

Experimental animals and xenograft model. Cells from one confluent 150-cm^2 Falcon cell culture flask per mouse with T47D-ERβ or normal T47D cells of the same clonal origin was diluted with 200 μL normal medium + 200 μL Matrigel (BD Falcon, San Jose, CA). The cell suspension

Note: Possible conflict of interest: J-Å. Gustafsson is cofounder, deputy board member, consultant, and shareholder of KaroBio AB.

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was injected into the mammary fat pad of 5-week-old pathogen-free SCID/beige mice (Taconic, Ry) on day 0. E_2 pellets, 0.72 mg/pellet (IRA, Sarasota, FL), were injected s.c. in the neck with pellet trochar (IRA). After 4, 8, 16, or 30 days, the mice were sacrificed and the tumor volume was measured with caliper according to the formula length × width × height. All tumors were fixed in 4% paraformaldehyde and stored in 75% ethanol. After this, tissue was paraffin-embedded and subsequently sliced into 4.5-μm sections according to standard protocol. Tumors of sufficient size at necropsy were divided into three similar parts for preparation of mRNA and protein as described below.

Western immunoblotting. Cells were plated and grown in 150-mm plates until 30% confluence was reached. At time point 0, the cells were incubated with E_2 with or without tetracycline. Harvesting and extraction were done at different times (0-36 hours) according to standard protocol. SDS-PAGE was done as described (18). The following primary antibodies were used: VEGF (Santa Cruz Biotechnology, Santa Cruz, CA), PDGFβ (BD Biosciences, San Jose, CA), Ki67 (DakoCytomation, Carpinteria, CA), CD31 (BD PharMingen, San Diego, CA), β-actin (Sigma), and ERβ (chicken antibody raised in our laboratory).

Immunohistochemistry. Antigen retrieval was done by microwave boiling in 0.01 mol/L citric acid (pH 6.0) or with Proteinase K solution for CD31 staining. Endogenous peroxidase activity was blocked with 0.5% hydrogen peroxide. Primary antibody was diluted 1:100 to 1:500 in 3% bovine serum albumin and 0.3% Triton X-100 (see above for antibody specifications) and incubated overnight at 4°C, followed by incubation with a biotinylated secondary antibody (1:200) in 0.3% Triton X-100 for 1 hour. Biotinylated secondary antibodies used were anti-mouse IgG and anti-rat IgG, both from Vector Laboratories (Burlingame, CA), incubated (1:200) in 0.3% Triton X-100 for 1 hour. Sections were finally incubated in the streptavidin-horseradish peroxidase ABC complex (Vector Laboratories) for 1 hour, stained in 3,3′-diaminobenzidine, and counterstained with Mayer hematoxylin (Sigma, Poole, United Kingdom) before dehydration through ethanol, and mounted in dibutyl phthalate xylene.

Measurement of microvessel density and counting of Ki67- and ERβ-positive cells. Microvessel density was calculated by first identifying the areas of highest vascularization with ×10 objective in the periphery of CD31 immunohistochemically stained tumor slides. Microvessel density was then determined by counting the number of vessels with ×20 objective in two independent fields per tumor. Vessels to be counted were identified by one of the following criteria: CD31 (mouse)–positive vascular formations or vascular formations containing luminal erythrocytes. Average microvessel density and SD of each group (±ERβ) was measured. Counting of ERβ/Ki67–positive cells on immunohistochemically stained slides was done by first identifying areas with most intensive staining. The number of Ki67- and ERβ-positive cells in two independent fields per tumor slide was counted with ×20 objective. Average number of stained cells and SD for each group (±ERβ) were calculated.

ELISA assay. ERβ-inducible T47D cells were incubated in estrogen-depleted RPMI medium containing 50 nmol/L ICI for 48 hours. Thereafter, medium was changed to normal RPMI medium ± E2, ±tetracycline, and cell cultures were placed in hypoxia chamber containing <1% O_2 at 37°C. After 72 hours, the conditioned cell medium was analyzed according to standard ELISA protocol (Quantakine human VEGF and PDGFβ; R&D Systems, Minneapolis, MN). RNA was extracted and purified from the T47D cells according to standard protocol.

Real-time PCR. RNA extraction and cDNA synthesis were done as described earlier (19). Real-time PCR was done with SYBR-Green PCR Master Mix or TaqMan Universal Mastermix (Applied Biosystems, Foster City, CA). The following primers and probes were used: *VEGF* forward: 5′-CTCTACCTCCACCATGCCAAGT-3′; reverse: 5′-TGATTCTGCCCTCCTCCTTCT-3′. *PDGFβ* forward: 5′-CTGCTACCTGCGTCTGGTCA-3′; reverse: 5′-CATCAAAGGAGCGGATCGA-3. *ERβ* forward: 5′-TCCATGCGCCTGGCTAAC-3′; reverse: 5′-CAGATGTTCCATGCCCTTGTTA-3. Probe: 5′-FAM (6-carboxyfluorescein)-TCCTGATGCTCCTGTCCCACGTCA(6-carboxytetramethylrhodamine)-3′. *18S rRNA* forward: 5′-CCTGCGGCTTAATTTGACTCA-3′; reverse: 5′-AGCTATCAATCTGTCAATCCTGTCC-3 as a reference gene. The real-time PCR reactions were done in an ABI PRISM 7500 (Applied Biosystems) under the following conditions: 50°C for 2 minutes, 95°C for 10 minutes, followed by 40 to 50 cycles at 95°C for 15 seconds and 60°C for 1 minute. The optimum concentration of primers was determined in preliminary experiments and all primer pairs were checked with melting curve analysis.

Cloning and transfections. The *VEGF* promoter (forward primer, 5′-AAATTCTTCTCCCCTGGGAA-3′; reverse primer, 5′-AATGAATATCAAATTCCAGCA-3′) and *PDGFβ* promoter (forward primer, 5′-CAGTGCAAGCGGAGGAGATGA-3′; reverse primer, 5′-CGGCTGCAGGAGGAGAAGTTG-3′) were amplified by PCR from human genomic DNA and subsequently subcloned into pGL3-basic luciferase vector (Promega).

Transfection of T47D cells was done by culturing cells on six-well cell culture dishes until 50% to 70% confluence was reached, whereupon the cells were synchronized. Transient transfections were done using Lipofectamine 2000 (Invitrogen) according to the protocol from the manufacturer. The reporter plasmid (*VEGF* or *PDGFβ* promoter 1.0 μg/well) was transiently cotransfected together with an expression plasmid (*ERβ* 0.5-0.1 μg) for 6 hours. When necessary, the total amount of DNA was completed with pcDNA3 (Invitrogen). After transfection, cells were treated with 10 nmol/L E_2 or ethanol in stripped medium for 24 or 48 hours. Cells were then lysed and luciferase activity was determined with a microplate luminometer (Berthold) using luciferase assay kit from Biothema. All data were normalized to β-galactosidase enzyme activity obtained after cotransfection with a β-galactosidase-lacZ plasmid (100 ng/well).

Hypoxia chamber experiments. T47D-ERβ cells were incubated for 48 hours in normal stripped medium without E_2 during normoxia. Cells were then incubated in 0.5% O_2 in 37°C for 48 hours in the presence of E_2. Cells were subsequently harvested with TRIzol reagent according to instructions from the manufacturer.

Statistics. Values are expressed as means with 95% confidence intervals. Tumor growth curves were constructed from the mean tumor volume at each time point of measurement. Unpaired, two-tailed *t* test was used to compare differences between two groups. One-way ANOVA analysis with Tukey's multiple comparison posttest was used to test differences between three or more groups. Significance is presented as $^{*}P < 0.05$; $^{**}P < 0.005$; $^{***}P < 0.001$; and NS, nonsignificant.

Results

Expression of ERβ reduces volume of T47D breast cancer xenografts. In ERα-positive breast cancer cells such as T47D or MCF7, E_2 treatment leads to increased proliferation and increased tumor growth when these cells are implanted into mice (20, 21). To study the effect of ERβ in this context, we have generated T47D breast cancer cells with stable Tet-Off–inducible ERβ expression. These cells are grown in the presence of tetracycline and should only express high levels of ERβ after removal of tetracycline. By orthotopic implantation of T47D-ERβ or parental T47D cells into SCID/beige mice, the effect of ERβ on ERα-induced tumor growth may be investigated. Preliminary studies with T47D-ERβ cells implanted into SCID/beige mice showed that there was some expression of ERβ even in the presence of tetracycline. Accordingly, tumor growth in the presence of tetracycline could not be used as baseline measure of ERα-controlled growth and parental T47D cells were used instead. T47D cells are E_2 dependent for their growth; therefore, at the start of the experiment, all animals were implanted with 60-day-release E_2 pellets. To confirm that ERβ was expressed in T47D-ERβ tumors, ERβ mRNA and protein levels were monitored by real-time PCR and immunohistochemical analysis. There was an extremely low, but nonetheless measurable, level of ERβ mRNA in T47D cells.

As shown in Fig. 1*A*, exogenous ERβ expression reduced tumor volume at end point 30 days by 80%. Of the eight mice implanted with T47D-ERβ, in three animals at necropsy, no tumor tissue could be found. Thirty days after implantation, levels of ERβ

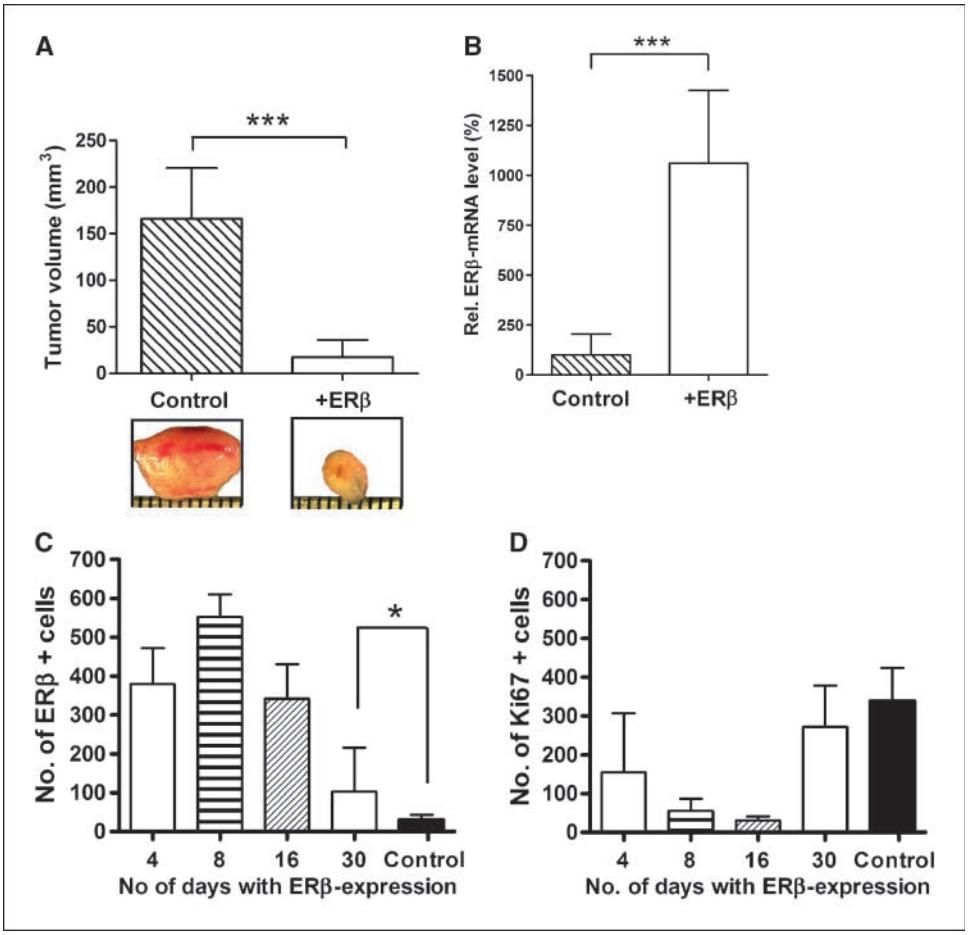

Figure 1. SCID/beige mice orthotopically transplanted with Tet-Off ERβ-inducible T47D cells or normal T47D cells as control. Both cells were of identical clonal origin. Mice were subsequently implanted with slow-release E_2 pellet. *A,* tumor volume of removed xenografts at end point 30 days. *Control column,* normal T47D xenograft ($n = 6$); *+ERβ column,* ERβ-overexpressing xenografts ($n = 8$). *Columns,* average volumes; *bars,* SD ($P = 0.0003$). *B,* ERβ-mRNA levels in the tumor xenografts above. *Columns,* average ($n = 6, 3$); *bars,* SD ($P = 0.0004$). Number of (*C*) ERβ-positive and (*D*) Ki67-positive cells in xenograft slides per visual field (×20 objective) identified by immunohistochemistry. *Column 1,* ERβ-expressing xenografts from mice sacrificed 4 days after implantation ($n = 4$); *columns 2, 3,* and *4,* xenografts 8 ($n = 4$), 16 ($n = 4$), and 30 days ($n = 6$) after implantation; *column 5,* T47D control xenografts ($P = 0.0421$ in *C*). Number of positive cells was counted in the two most intensively stained areas on each xenograft slide. *Columns,* average; *bars,* SD. Significance is presented as *, $P < 0.05$; ***, $P < 0.001$.

mRNA in T47D-ERβ xenographs were >10-fold higher than in the T47D xenografts (Fig. 1*B*). Immunohistochemical detection of ERβ in paraffin-embedded tumor slides showed strong expression of ERβ 4 to 16 days after implantation. There was a gradual decline in expression so that by day 30, there was only a small but statistically significant difference in ERβ expression between the T47D-ERβ and the T47D tumors ($P = 0.0421$; Fig. 1*C*).

As expected, the Ki67 proliferation index was decreased in the ERβ-expressing tumors, and at all time points Ki67 was inversely associated with ERβ expression (Fig. 1*D*). As the number of ERβ-expressing cells decreased after day 16, the number of Ki67-positive cells increased. Representative pictures of ERβ and Ki67 immunohistochemically stained slides are shown in Fig. 2.

ERβ expression correlates to reduced microvessel density. The supply of oxygen and nutrients provided by angiogenesis is necessary for tumor growth, and the microvessel density is a strong predictor of aggressive disease (13). To investigate whether ERβ influenced angiogenesis in the tumors, endothelial cells were

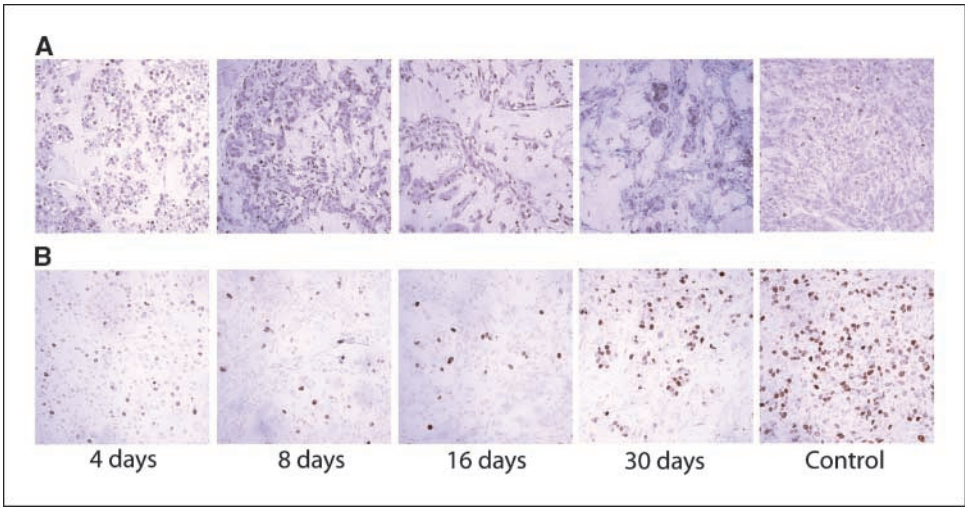

Figure 2. Representative pictures of (*A*) ERβ and (*B*) Ki67 immunohistochemically stained slides from the xenografts analyzed in Fig. 1*C* and *D*.

visualized with an antibody against mouse CD31. These immunohistochemical studies revealed that ERβ expression correlated significantly to reduced microvessel density (Fig. 3*A*).

Angiogenesis is dependent on growth factors and their receptors. The most important factors in tumor angiogenesis are VEGF, bFGF, PDGFβ, and angiopoietins (22, 23). These factors are also up-regulated in high-stage and high-grade human tumors (24). Therefore, we investigated the expression of these factors in the T47D-ERβ and T47D xenografts. By Western blot analysis of tumor lysates, we found that the total amount of VEGF and PDGFβ proteins was decreased in the ERβ-expressing tumors (Fig. 3*B*). Due to the very small volume of the ERβ-expressing tumors, proteins could not be extracted from all tumors. Real-time PCR analysis of tumor xenografts from 30 days ± ERβ expression showed no significant difference in VEGF mRNA but a strong inhibition of PDGFβ-mRNA by ERβ expression ($P = 0.003$; Fig. 3*C*). In Fig. 3*D*, representative immunohistochemical pictures are shown.

ERβ decreases expression of VEGF and PDGFβ in T47D-ERβ cells. We investigated whether ERβ was directly involved in regulating the production of VEGF and PDGFβ or whether the induction of these factors was induced by hypoxia independent of the presence of ERβ. We incubated ERβ-inducible T47D cells *in vitro* under normoxic conditions (20% O_2) in presence of E_2. Expression of ERβ reduced VEGF mRNA as well as in secreted form under both normoxic and hypoxic conditions (Fig. 4*A* and *B*). PDGFβ was strongly down-regulated at the mRNA level and at secreted protein level as a result of ERβ expression, both under normoxic and hypoxic conditions (Fig. 4*C* and *D*). To further investigate this transcriptional regulation, cells were transiently transfected with promoter constructs of VEGF and PDGFβ. E_2 treatment resulted in an increase in promoter activity with both constructs (Fig. 5), caused by ligand activation of ERα. Cotransfection of an ERβ expression vector resulted in reduction of VEGF and PDGFβ promoter activities in the presence of E_2.

Discussion

We have previously shown that expression of ERβ in cultures of T47D cells opposes ERα-mediated cell cycle progression by interference with cell cycle factors such as cyclin E and Cdk2 (7).

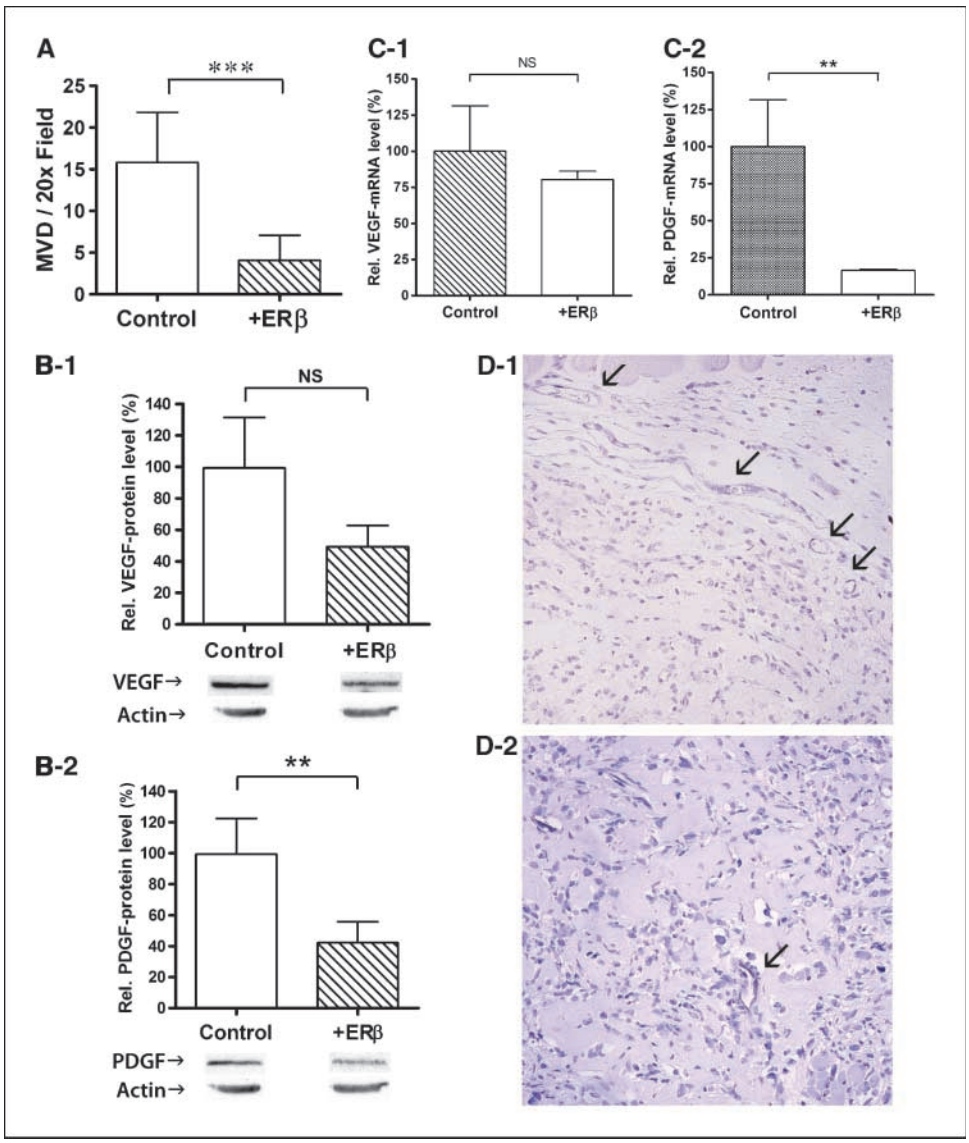

Figure 3. Inhibition of tumor angiogenesis by ERβ expression at 30 days. *A,* microvessel density (*MVD*) in control T47D xenografts ($n = 6$) and ERβ-overexpressing xenografts ($n = 5$). Animals were sacrificed at end point 30 days. Microvessel density was measured as the number of CD31-positive vessels per visual field (×20 objective) identified by immunohistochemistry of xenografts. Number of vessels were counted in the two most intensively stained areas on each xenograft slide. *Columns,* average; *bars,* SD ($P < 0.0001$). Lysates from these xenografts showing average (*B-1*) VEGF ($n = 6, 3$; $P = 0.0723$) and (*B-2*) PDGFβ protein levels ($n = 6, 3$; $P = 0.006$). *Columns,* average; *bars,* SD. Lysates were analyzed by absorbance of Western blot membranes (Bio-Rad software). Data were based on VEGF or PDGFβ/β-actin density of each sample with representative Western blot. mRNA expression of VEGF (*C-1*; $n = 6, 3$; $P = 0.34$) and PDGFβ (*C-2*; $n = 6, 3$; $P = 0.003$) in tumor xenografts, analyzed with real-time PCR. *Columns,* average; *bars,* SD. Representative immunohistochemical pictures of control xenograft (*D-1*) and ERβ-overexpressing xenograft (*D-2*) analyzed in (*A*). *Arrows in D-1,* CD31-stained endothelium. Significance is presented as **, $P < 0.005$; ***, $P < 0.001$; *NS*, non-significant differences.

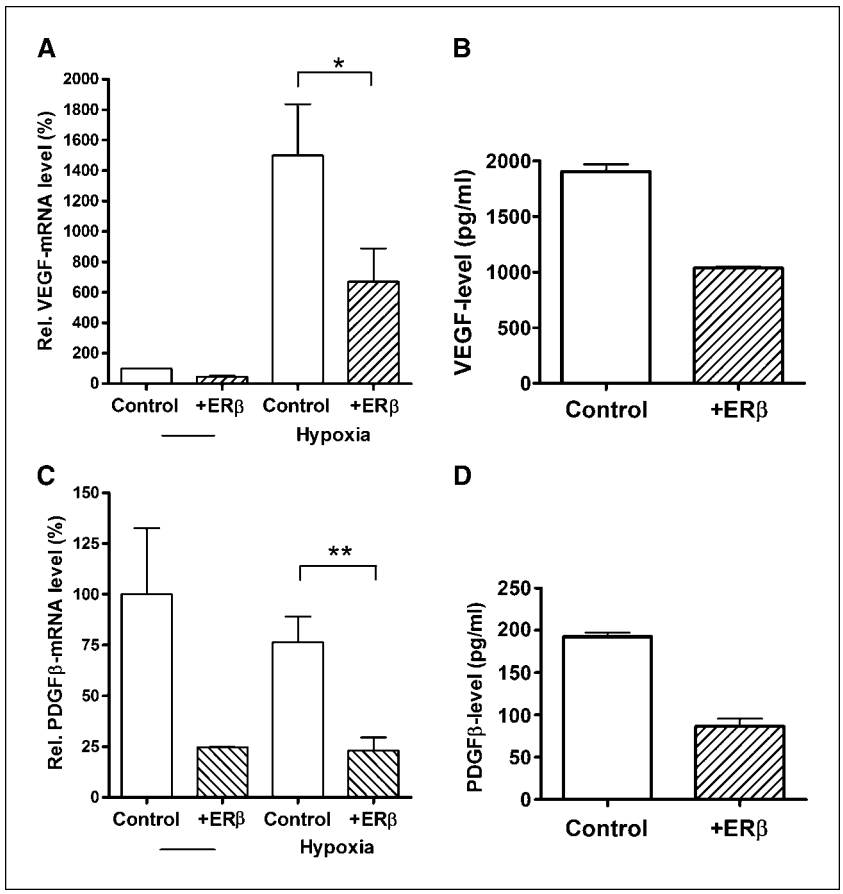

Figure 4. Inhibition of growth factor expression *in vitro*. mRNA expression in ERβ-inducible T47D breast cancer cells incubated with E_2 ± hypoxia (<1% O_2) ± tetracycline for 24 hours, and protein levels in cell culture medium after 72 hours incubation with E_2 + hypoxia (<1% O_2) were analyzed with ELISA assay. *A,* VEGF mRNA ($P = 0.0232$, *columns 3-4*). *B,* VEGF protein level ($P < 0.0001$) in cell culture medium, (*C*) PDGFβ mRNA ($P = 0.0028$, *columns 3-4*), and (*D*) PDGFβ protein level ($P = 0.0048$) in cell culture medium. *Columns* (*A* and *C*), average of triplicates; (*B* and *D*), average of at least two independent experiments; *bars,* SD. Significance is presented as *, $P < 0.05$; **, $P < 0.005$.

The purpose of the present study was to determine whether the observations made in cell culture would apply to breast cancer cells *in vivo*. As shown by others using viral transfection systems, ERβ inhibits tumor formation of MCF-7 cells in a mouse model (8). One critique of viral transfection studies is that uncontrolled overexpression of the transfected gene leads to unphysiologically high protein levels. With the use of a Tet-Off-system, we here report that when ERβ expression is induced in breast cancer xenografts, tumor growth is inhibited. Furthermore, the proliferation marker Ki67 was inversely associated with ERβ in most groups; when the number of ERβ+ cells was high, the number of Ki67+ cells was low, whereas when the number of ERβ+ cells was low, the number of Ki67+ cells was high (Fig. 1*C* and *D*). This finding gives further support to the notion that ERβ is antiproliferative.

There was a decrease in the number of ERβ+ tumor cells from days 16 to 30 in the T47D-ERβ group (Fig. 1*C*), in spite of strong induction of ERβ at mRNA level. The reason for this loss is not known but a possible mechanism could be increased ERβ protein degradation. This would also be in line with the reports showing decreased ERβ levels in breast cancer. Studies of the mechanisms behind the observed decrease in ERβ levels in the transplanted tumor cells are ongoing.

One possible explanation for the reduction in tumor volume by ERβ expression could be the influence of ERβ on angiogenesis. We found that in the T47D-ERβ xenografts, microvessel density was significantly lower than in the parental T47D tumors (Fig. 3*A*). As expected, PDGFβ was reduced on both mRNA and protein level in the T47D-ERβ xenografts. We could see no reduction of VEGF-mRNA in xenografts by ERβ expression but a nonsignificant trend toward a reduction of VEGF protein ($P = 0.0723$; Fig. 3*B* and *C*).

Tamoxifen, which is known to activate ERβ in certain promoter contexts, has been shown to inhibit growth of breast cancer xenografts by affecting angiogenesis (25). Because oxygen tension is a major stimulator of angiogenesis, the question was whether the lower microvessel density in the ERβ-expressing tumors was simply because the tumors were smaller and less hypoxic. We therefore analyzed the expression of the three most important proangiogenic factors, namely VEGF, bFGF, and PDGFβ, during normoxic as well as hypoxic conditions *in vitro*. Secreted bFGF protein was not detectable in the cell culture supernatants but both PDGFβ and VEGF were reduced by ERβ expression (Fig. 4).

It has earlier been reported that ERα increases the expression of PDGFβ, bFGF, and VEGF, and that this induction of growth factors might be an important mechanism explaining how ERα stimulates breast cancer growth and progression. The role of ERβ in regulation of PDGFβ is not clear; furthermore, there are conflicting data as to how ERβ affects VEGF expression in tumors (26, 27).

In transient transfections with a 1,026 bp VEGF promoter construct, a reduction in promoter activity by cotransfection with ERβ was seen in the presence of E_2. Accordingly, ERβ might be a direct transcriptional inhibitor of VEGF, opposing the effect of ERα. E_2 treatment in the absence of ERβ cotransfection showed only a minor up-regulation of the VEGF promoter activity. Earlier reports with longer promoter constructs have shown strong

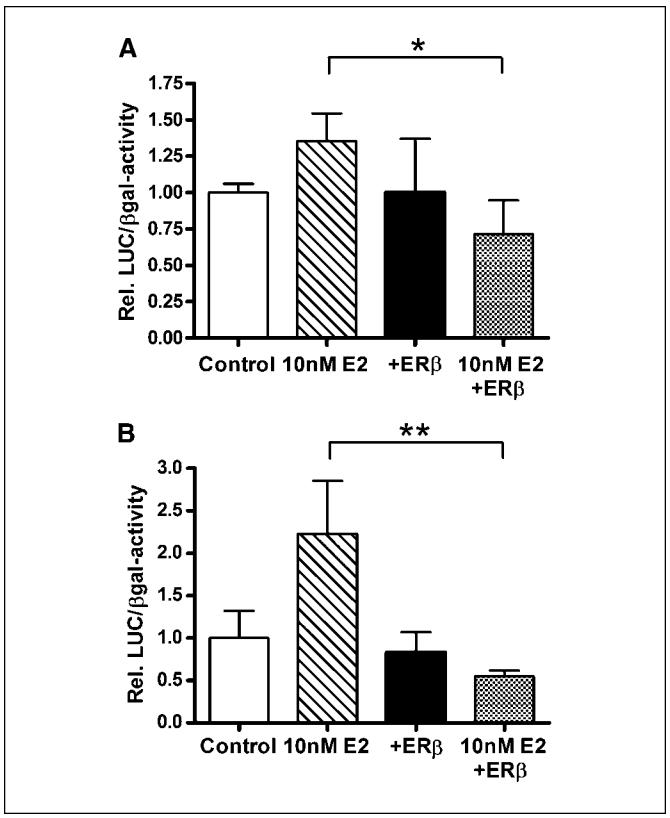

Figure 5. Transient transfections of T47D cells with (*A*) pGL3-VEGF-LUC (−881/+145; $P = 0.0211$, *columns 2-4*) and (*B*) pGL3-PDGFβ-LUC (−981/+25; $P = 0.0096$, *columns 2-4*) ± cotransfection with ERβ expression vector ± E_2. All data were normalized to β-galactosidase enzyme activity obtained after cotransfection with a β-galactosidase-lacZ plasmid. *Columns,* average of triplicates; *bars,* SD. All experiments were repeated with similar results. Significance is presented as *, $P < 0.05$; **, $P < 0.005$.

activation by ERα (28). VEGF is well known for its activation of endothelial cells, resulting in their proliferation and migration. In addition, as shown in a recent report (29), VEGF influences breast cancer cell survival and growth by direct effects on cancer cell surface VEGFR-2 in an autocrine fashion.

Transient transfections with 1,006 bp PDGFβ promoter constructs also showed inhibition by ERβ. PDGFβ is a growth factor with wide potentials. In addition to its proangiogenic effects, PDGF family members stimulate proliferation of breast cancer cells (30). Also, PDGFβ have been reported to induce aromatase activity in T47D cells (31). Therefore, it is an interesting possibility that induction of ERβ would actually inhibit the local production of E_2 in breast tumors, in the same way as pharmaceutical aromatase inhibitors do. Reduction of growth factor expression could provide one explanation as to how ERβ inhibits tumor growth. Furthermore, preliminary experiments done by our group indicate that ERβ correlates inversely to PDGFβ and VEGF mRNA in purified breast cancer cells from 14 different human breast tumors. This will be investigated in more detail with increased sample size.

Altogether, our results indicate an antitumorigenic role of ERβ in breast cancer. This makes ERβ an interesting therapeutic target in breast cancer and perhaps treatment with ERβ-selective ligands might work as both antiproliferative and antiangiogenic therapy.

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