# The Role of Amphiregulin in Exemestane-Resistant Breast Cancer Cells: Evidence of an Autocrine Loop

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## Abstract

**Exemestane-resistant breast cancer cell lines (i.e., ExeR), derived from MCF-7 cells expressing a high level of aromatase (MCF-7aro), were generated in our laboratory. The epidermal growth factor (EGF)–like protein amphiregulin (AREG) was highly expressed in ExeR cells based on cDNA microarray analysis. The high levels of AREG mRNA in ExeR cell lines were confirmed by real-time reverse transcription-PCR. The high levels of AREG protein in ExeR cell lysates and culture media were confirmed by Western blot analysis and ELISA, respectively. Furthermore, our Western blot analysis showed that whereas no AREG was detected in the DMSO control, overnight treatment of parental MCF-7aro cells with 1 μmol/L exemestane strongly induced the expression of AREG. This induction was totally blocked by 100 nmol/L of pure anti-estrogen ICI 182,780, implying estrogen receptor (ER) dependence of exemestane-induced AREG expression. MCF-7aro cells were not able to proliferate in hormone-free medium, but were able to proliferate in conditioned medium from ExeR cells, similar to the treatment of recombinant human AREG. Small interference RNA targeting AREG inhibited ExeR proliferation, confirming that AREG is truly functioning as a growth factor of ExeR cells. The specific inhibitors to ER (ICI 182,780), EGF receptor (EGFR; AG1478), and mitogen-activated protein kinase (MAPK; U0126) all showed dose-dependent suppression of the proliferation of ExeR cells, indicating the involvement of the ER, EGFR, and MAPK pathways. Based on these findings, we propose a possible mechanism that underlies exemestane resistance: exemestane induces AREG in an ER-dependent manner. AREG then activates the EGFR pathway and leads to the activation of the MAPK pathway that drives cell proliferation.** [Cancer Res 2008;68(7):2259–65]

## Introduction

Exemestane, letrozole, and anastrozole are Food and Drug Administration–approved aromatase inhibitors. Aromatase inhibitors are proved to be very effective in treating hormone-dependent breast cancer in postmenopausal women (1). However, for prolonged treatment, resistance becomes a major concern. To study the mechanisms of aromatase inhibitor resistance, our lab has developed several aromatase inhibitor–resistant cell lines that are derived from MCF-7 cells that overexpress aromatase (MCF-7aro). These resistant cell lines were selected under the following conditions: testosterone plus letrozole (T + LetR), testosterone plus anastrozole (T + AnaR), anastrozole only (AnaR), testosterone plus exemestane (T + ExeR), exemestane only (ExeR), or long-term estrogen deprivation (LTEDaro). MCF-7aro cells cultured in testosterone (in which testosterone was converted to 17β-estradiol) were used as positive controls (2, 3).

Although they all target aromatase specifically and potently, it is known that different aromatase inhibitors behave differently based on their structures. Letrozole and anastrozole are nonsteroidal inhibitors and interact with the heme group. Exemestane is a steroidal inhibitor and is also known as a "mechanism-based" aromatase inactivator. Exemestane binds to aromatase irreversibly and causes time-dependent aromatase inactivation (4). A recent study from our lab showed that exemestane could destabilize aromatase protein, in addition to inhibiting its activity (5).

As an unbiased and systemic approach that could give important information about the resistance mechanisms of different aromatase inhibitors, our laboratory has generated three to six replicates of resistant cell lines to each aromatase inhibitor and examined the gene expression profiles using cDNA microarray analysis. From our analysis, we have noticed high levels of amphiregulin (AREG) expression in ExeR cells. Because the expression of AREG is known to be up-regulated by estrogen (6, 7), we were not surprised to find that the expression of AREG was high in MCF-7aro cells treated with testosterone and was detected in all testosterone + aromatase inhibitor–treated cells (T + LetR, T + AnaR, and T + ExeR) but not in AnaR and LTEDaro cells. Whereas the microarray analysis will be discussed in detail in a separate article, this present study will be focused on AREG expression in ExeR cells.

AREG was originally isolated from conditioned medium of phorbol 12-myristate 13-acetate (PMA)–stimulated MCF-7 cells (8). AREG caught our attention for the following reasons (*a*) AREG is an epidermal growth factor (EGF)–like growth factor that binds to and activates EGF receptor (EGFR; ref. 9); (*b*) AREG was reported to be overexpressed in many types of cancers including colorectal, lung, prostate, and breast cancers (10); and (*c*) AREG is an estrogen-regulated gene (6, 7). What was really unexpected from the results of our microarray experiments was the high level of AREG in ExeR cells (i.e., cells treated with exemestane only), in contrast to undetectable expression in AnaR cells. Because the only difference between ExeR and AnaR is the added drugs (exemestane and anastrozole, respectively), it is a strong indication that the induction of AREG is due to some unique characteristics of exemestane.

MCF-7 selected under long-term estrogen deprivation (LTED) was used as a model for aromatase inhibitor resistance by several groups (11). This is a relatively well-characterized model, and AREG was not reported as a mitogenic factor in this model. The LTEDaro cells in our lab were similarly developed (MCF-7aro cells selected under hormone-free, inhibitor-free conditions over 6 months). Because the AREG level is very low in LTEDaro cells, LTEDaro cells were used as a control in studying the effect of

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exemestane after long-term culture of MCF-7aro in the presence of exemestane only. We first confirmed the exemestane-induced expression of AREG before showing the growth-stimulating effect of AREG and providing evidence that the exemestane-induced AREG expression is contributing to exemestane resistance in our model system.

## Materials and Methods

**Drugs and inhibitors.** The following compounds and reagents were used in this study: recombinant human AREG (R&D Systems), AG1478 (Calbiochem), U0126 (Promega), testosterone (Sigma), ICI 182,780 (ICI; Tocris), and exemestane (Pfizer). 1β-$^3$H-Androstenedione was purchased from Perkin-Elmer.

**Cell culture and proliferation assay.** All cell culture media and supplements are from Invitrogen. The MCF-7aro cell line was generated by Zhou et al. (12). We have generated three independent ExeR lines. All three lines were maintained in phenol red–free MEM supplemented with 10% charcoal dextran–treated fetal bovine serum (FBS) and 1 μmol/L exemestane. All of these cells were well adapted in the presence of exemestane. Elevated AREG levels were detected in all three lines by microarray analysis and subsequent reverse transcription-PCR (RT-PCR), indicating that this was not due to a single clone effect. Therefore, only one of the ExeR lines was used in this study.

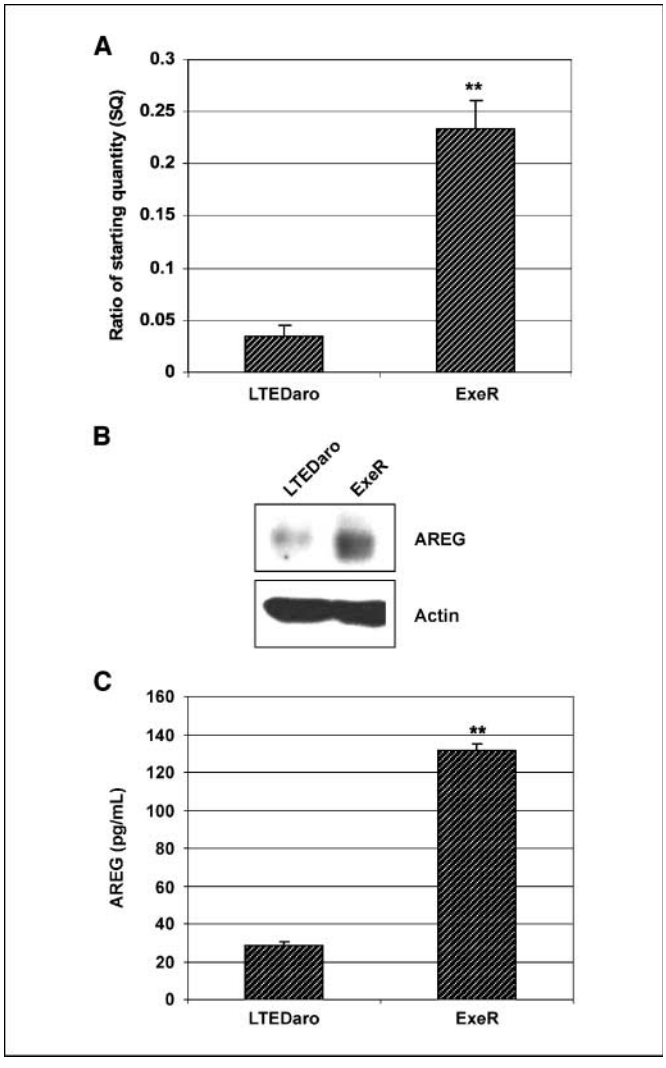


**Figure 1.** High levels of AREG expression in ExeR cells. AREG expression levels in ExeR cells were compared with those in LTEDaro cells by real-time RT-PCR (*A*; results normalized to β-actin), Western blot (*B*), and ELISA of culture media (*C*). *Columns,* mean; *bars,* SD. **, $P < 0.01$, compared with LTEDaro.

MCF-7aro cells were cultured in MEM supplemented with 10% FBS, 110 mg/L sodium pyruvate, 100 μmol/L nonessential amino acids, and 100 mg/L G418. LTEDaro cells were cultured in phenol red–free MEM supplemented with 10% charcoal dextran–treated FBS, 110 mg/L sodium pyruvate, and L-glutamate. T + ExeR cells were cultured in the same medium as for LTEDaro, but supplemented with 1 nmol/L testosterone or 1 μmol/L exemestane, respectively. For preparation of conditioned medium, ExeR cells were cultured in their maintenance medium but without exemestane for 3 d, then supernatant was collected and used as conditioned medium. For proliferation assay, cells grown in 60-mm dishes were preconditioned in hormone-free, phenol red–free medium for 24 h before assay. Cells were then detached with phenol red–free TrypLE and replated into a 96-well plate. After treatment for 5 d, 20 μL of Cell Titer96 AqueousOne (Promega) were added into each well containing 100-μL culture medium. The plates were incubated at 37°C for 1 h and then read with a Molecular Devices SpectraMax M5 plate reader at $A_{490}$ nm.

**Western blot analysis.** Cells grown in 60-mm dishes were lysed in 300-μL SDS lysis buffer [62.5 mmol/L Tris-HCl (pH 6.8), 2% w/v SDS, 10% glycerol, 50 mmol/L DTT, 0.01% bromophenol blue]. Sixty micrograms of lysate were resolved on a 12% SDS-PAGE gel and then transferred onto a polyvinylidene difluoride membrane (Millipore). The membrane was blocked in blocking buffer (LI-COR) for 1 h at room temperature and then with anti-AREG goat antibody (R&D Systems) at 4°C overnight. Secondary horseradish peroxidase–conjugated rabbit anti-goat antibody was from Santa Cruz Biotechnology. Anti–estrogen receptor (ER)-α mouse antibody was also from Santa Cruz Biotechnology. Detection was done with Western PICO system (Pierce). AREG has been shown to be first produced as a membrane-anchored glycoprotein, and then secreted through a metalloproteinase-dependent process (13). Due to the variations in glycosylation and protease cleavage sites, multiple forms varying in size from 9 to 60 kDa were reported (10, 13). The 25-kDa form is the predominant form from whole-cell lysates. The 25-kDa form was found in this study.

**Aromatase activity assay.** Aromatase activity was measured by the conversion of 1β-$^3$H-androstenedione into estrone and tritiated water. For each reaction, 5 μg of placental microsome were incubated at 37°C for 20 min in a 0.5-mL solution containing 67 mmol/L potassium phosphate (pH 7.4), 0.1% bovine serum albumin, 0.01 mmol/L progesterone, 0.3 mmol/L NADPH, and 0.1 mmol/L 1β-$^3$H-androstenedione. Exemestane at 1 μmol/L, ICI at 100 nmol/L, or their combinations were added into designated vials. The reactions were stopped by adding 60 μL of 20% tricholoroacetic acid. The reaction mixture was then extracted twice with charcoal pellets, and 200 μL of the tritiated water containing supernatant were added to 3 mL of scintillation liquid and counted with a liquid scintillation counter (LS-6500, Beckman Coulter, Inc.).

**Real-time RT-PCR.** Trizol reagent (Invitrogen) was used for total RNA isolation. All the reagents and instruments used for cDNA preparation and RT-PCR reaction were from Bio-Rad. Two micrograms of total RNA and iScript kit were used for cDNA preparation. Primers used were ordered from IDT, Inc.: 5′TGGATTGGACCTCAATGACA3′ and 5′AGCCAGGTATTAGCCAGGTATTTGTGGTTCG3′ for AREG; 5′AGAAGGAGATCACTGCCCCCCTGGCACC3′ and 5′CCTGCTTGCTGATCCACATCTGCTG3′ for β-actin. Annealing temperature for PCR was 63°C. SYBR Green Supermix and iCycler iQ5 Real-time PCR Detection System (Bio-Rad) were used.

**Small interference RNA assay and subsequent ELISA, proliferation assay, and real-time RT-PCR.** The following small interference RNA (siRNA) duplex sequences that target two different regions of AREG were referred from Gschwind et al. (14): CCACAAAUACCUGGCUATAdTdT and AAAUCCAUGUAAUGCAGAAdTdT. These duplexes were ordered from IDT. A scrambled sequence of duplex 1, CATCAGACACTGCTATACAdTdT, ordered from Dharmacon Research, was used as a negative control. The negative control was confirmed free of obvious targets in human using BLAST tool. All reagents for transfection were from Invitrogen, and assay

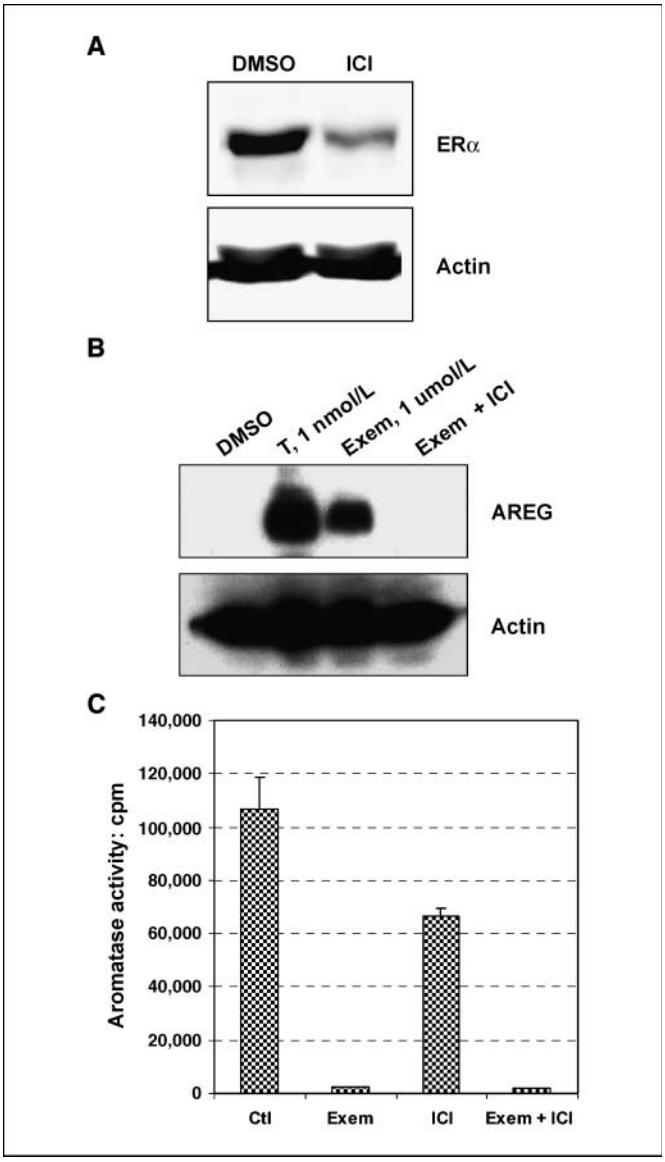


**Figure 2.** ER dependence of exemestane-induced AREG expression. *A,* MCF-7aro cells were cultured in hormone-free, phenol red–free medium supplemented with 1 nmol/L testosterone. Then cells were treated with 100 nmol/L ICI or DMSO for 7 h. *B,* MCF-7aro cells were cultured in hormone-free, phenol red–free medium for 24 h, followed by drug treatment overnight [testosterone (*T*) at 1 nmol/L, exemestane (*Exem*) at 1 μmol/L, and ICI at 100 nmol/L]. Cells were then lysed and Western blot analysis was done. *C,* aromatase activity was measured by the tritiated water release method and expressed as counts per minute (*cpm*). Exemestane at 1 μmol/L and ICI at 100 nmol/L were used. DMSO was used in control (*Ctl*). Placental microsome was used as source of aromatase.

was done per manufacturer's instructions. Briefly, cells were plated into a 24-well plate (in 500-μL medium per well) 1 d before transfection and were ~70% confluent at time of transfection. Oligofectamine and siRNA mixture was made with OptiMEM, and 100 μL of the mixture were added into each well. There were roughly 1.3 μg of siRNA duplexes and 3 μL of Oligofectamine per 100-μL mixture per well. Five days after transfection, 50 μL of culture medium per well were collected for ELISA (R&D Systems). For proliferation assay, 30 μL of Cell Titer96 AqueousOne (Promega) were added into each well and incubated for 1 h. One hundred microliters of the supernatant were then transferred into a 96-well plate for $A_{490}$ measurement. Finally, the cells were lysed in Trizol and RNA was prepared and real-time RT-PCR assay done to measure the AREG mRNA level.

**Statistical analysis.** All experiments were done in triplicate. Results were analyzed using the two-tailed *t* test (Microsoft Excel).

## Results

**AREG is highly expressed in ExeR cells.** Microassay analysis has shown high levels of AREG expression in ExeR cells. In Fig. 1*A* to *C*, AREG overexpression was confirmed by real-time RT-PCR, Western blot analysis of cell lysates, and AREG ELISA of the cell culture medium, respectively. In all three assays, results from ExeR cells were compared with those from LTEDaro cells (a negative control).

**Exemestane induces AREG expression in an ER-dependent manner.** Parental MCF-7aro cells were used to determine if high levels of AREG in ExeR cells were due to the presence of exemestane. ICI is known as a pure antiestrogen that degrades ER (15). Here we first checked the effect of ICI on ERα protein. There are two types of ER: ERα and ERβ. Our microarray analysis

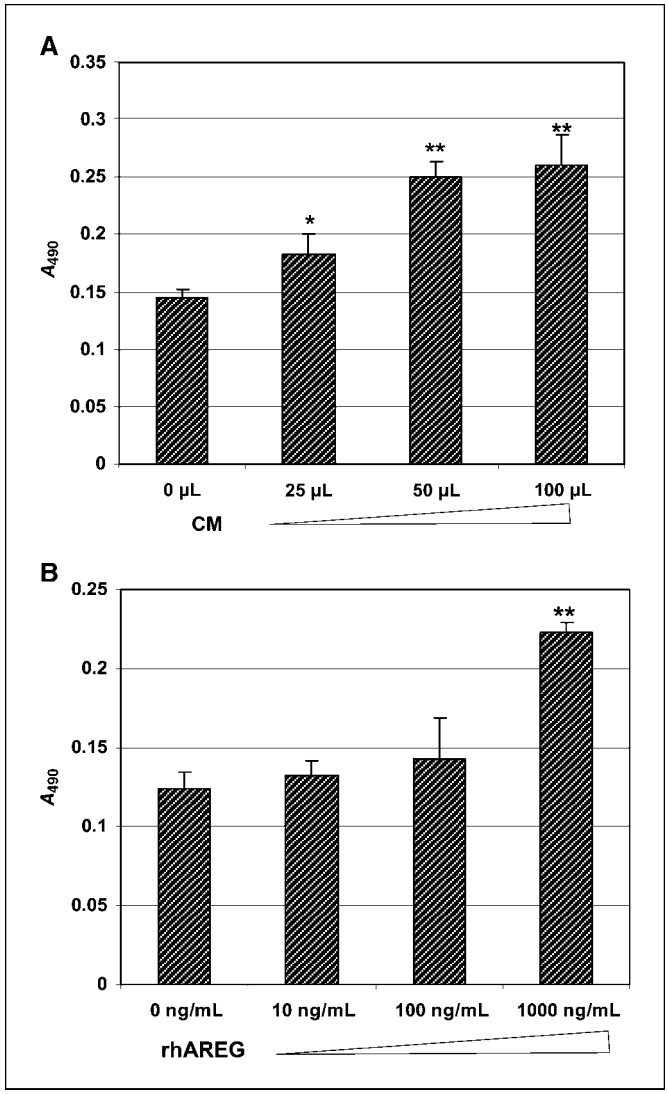


**Figure 3.** MCF-7aro proliferation assay. Proliferation of MCF-7aro cells was examined with the addition of conditioned media (*CM*) from ExeR cells (*A*) and recombinant human AREG (*rhAREG; B*). *Columns,* mean; *bars,* SD. *, $P < 0.05$; **, $P < 0.01$, compared with 0 μL conditioned medium or 0 ng/mL recombinant human AREG.

revealed that there is minimal expression of ERβ in MCF-7aro cells. Levels of ERα in MCF-7aro cells were significantly reduced after being treated with 100 nmol/L ICI for 7 hours (Fig. 2*A*). A similar effect of ICI on ER protein degradation in MCF-7 cells was reported by Jensen et al. (16). Next, the roles of exemestane and ER in AREG expression were determined (Fig. 2*B*). MCF-7aro cultured in hormone-free condition for 2 days had no detectable AREG. The expression of AREG was strongly induced by overnight treatment with 1 nmol/L testosterone. Exemestane at 1 μmol/L had a similar effect as 1 nmol/L testosterone, although the AREG level was clearly lower. Because AREG is known as an estrogen-regulated gene, it was examined if the exemestane-induced AREG was also ER dependent. The addition of 100 nmol/L of pure ER antagonist ICI totally blocked exemestane-induced AREG expression. Given the ability of ICI to degrade ER protein, we believe that the effect of ICI to abrogate exemestane-induced AREG expression is mediated through degradation of ER. However, to rule out any possible direct antagonism of ICI on exemestane, we carried out an experiment to determine whether ICI could prevent the inhibition of aromatase by exemestane. We showed that the aromatase activity from placental microsome was inhibited by 95% with 1 μmol/L exemestane. ICI at 100 nmol/L did not affect the inhibitory effect of exemestane (Fig. 2*C*). We believe that this experiment dismissed the possibility that ICI-aromatase interaction caused the inhibition of exemestane-induced AREG expression.

**Conditioned medium from ExeR cells or recombinant human AREG can stimulate MCF-7aro proliferation.** After confirming the high levels of AREG produced by ExeR cells, it was hypothesized that these ExeR cells cultured under hormone-free conditions produced AREG that could be secreted into the culture medium. This conditioned medium should be able to stimulate the proliferation of parental MCF-7aro cells, which normally would not proliferate in hormone-free medium. Adding increasing amounts of this conditioned medium to MCF-7aro cells cultured in hormone-free medium increased the proliferation rate accordingly (Fig. 3*A*). Increase of cell proliferation was also observed in MCF-7aro cells treated with increasing amounts of recombinant human AREG (Fig. 3*B*). These results support the hypothesis that the proliferation-stimulating effect of conditioned medium is caused by AREG.

**Knockdown of AREG with siRNA results in inhibition of ExeR cell proliferation.** To further define the role of AREG, siRNA was used to knock down AREG expression specifically. For reagent controls, a scrambled RNA was used. Again, LTEDaro cells were used as the negative control. Figure 4*A* shows the specific effect of AREG siRNA; it suppressed the levels of AREG mRNA in ExeR cells by >80%. LTEDaro cells had much lower levels of AREG; still, the inhibitory effect of siRNA was clear. Figure 4*B* shows the lowered levels of AREG protein measured by ELISA in culture supernatant of siRNA-treated cells. Finally, as shown in Fig. 4*C*, we have found a repressed proliferation of AREG siRNA–treated ExeR cells. In contrast, the proliferation rate of LTEDaro cells was not affected by siRNA, indicating that their proliferation is not modulated by AREG.

**ExeR cell proliferation is sensitive to inhibitors of ER, EGFR, or mitogen-activated protein kinase.** Exemestane-induced AREG expression was found to be ER dependent (Fig. 2); therefore, it was speculated that the proliferation of ExeR cells should be sensitive to antiestrogen. In addition, AREG exerts its mitogenic effect through binding and activation of EGFR and the subsequential activation of the mitogen-activated protein kinase (MAPK) pathway. ExeR cell proliferation might also be sensitive to inhibitors of EGFR or MAPK. AG1478 (EGFR inhibitor) and U0126 [MAPK/extracellular signal–regulated kinase kinase (MEK) inhibitor] were shown to be inhibitory on ERE-reporter assay and colony survival in HER2-overexpressing MCF-7 cells (17). In this set of proliferation assays, we examined antiestrogen ICI, anti-EGFR AG1478, and anti-MEK U0126 (Fig. 5*A*–*C*, respectively). All three

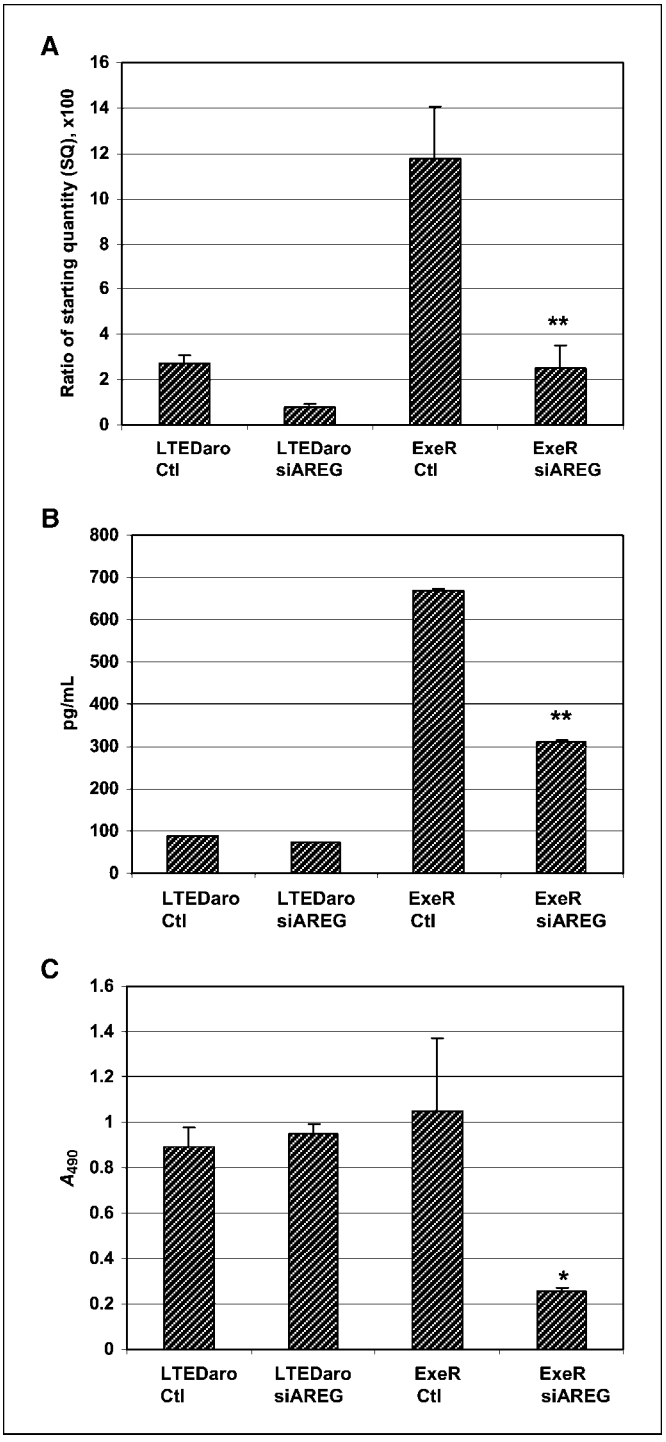


**Figure 4.** Block of ExeR cell proliferation with AREG siRNA. All experiments were done on both LTEDaro cells and ExeR cells. The effective repression of AREG expression was examined by real-time RT-PCR (*A*) and ELISA (*B*) of AREG in culture media. *C,* effect of siRNA on cell proliferation. *Columns,* mean; *bars,* SD. *, $P < 0.05$; **, $P < 0.01$, compared with ExeR control.

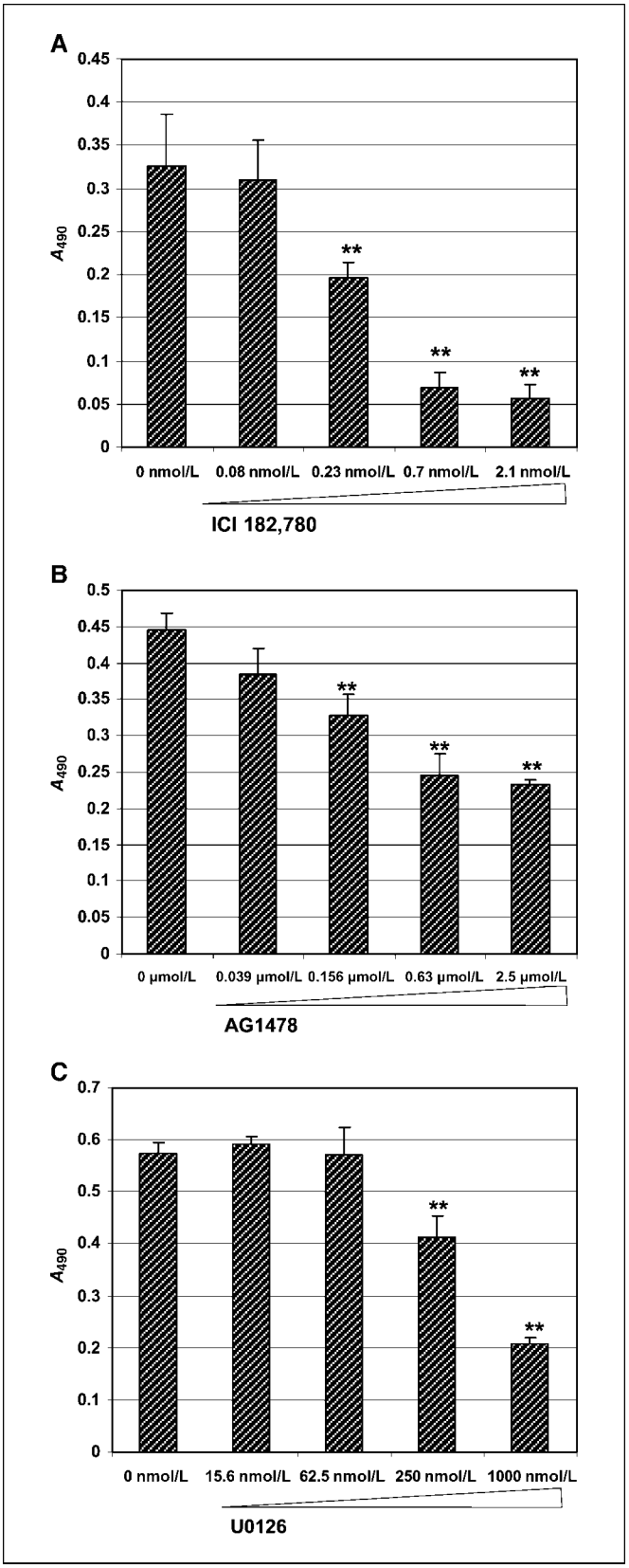


**Figure 5.** Suppression of the proliferation of ExeR cells by inhibitors of ER, EGFR, or MAPK. Proliferation assay was done on ExeR cells treated with pure antiestrogen ICI (*A*), specific EGFR inhibitor AG1478 (*B*), or MAPK kinase inhibitor U0126 (*C*). *Columns,* mean; *bars,* SD. **, $P < 0.01$, compared with control (0 nmol/L or 0 μmol/L).

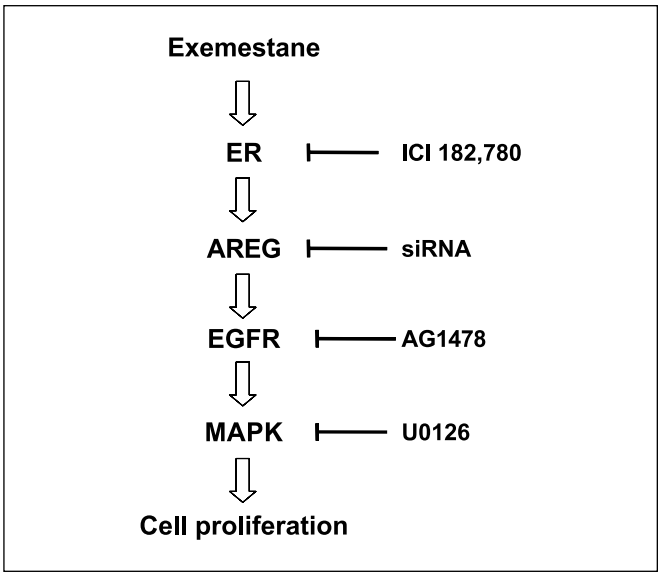


**Figure 6.** Proposed mechanism of AREG-mediated exemestane resistance.

drugs showed dose-dependent repression to ExeR proliferation. The high sensitivity to ICI strengthened the crucial role of ER in the proliferation of ExeR cells.

## Discussion

The aromatase inhibitor resistance study was started from gene expression profiling analysis of our aromatase inhibitor–resistant cell lines. Because the resistant cells were maintained in hormone-restricted or hormone-free conditions, an up-regulation of genes associated with or of the nature of growth factors that may overcome the normally required hormone dependency was expected. AREG turned out to be such a factor. Our microarray data showed high levels of AREG in ExeR cells, but not in LTEDaro or AnaR cells. These results were confirmed by real-time RT-PCR. Furthermore, we confirmed the high levels of AREG protein in ExeR cell lysates and in cell culture supernatants by Western blot analysis and ELISA, respectively. Using this ExeR model system, the essential roles of AREG and ER in exemestane resistance were shown. The essential role of AREG in ExeR cell proliferation was further defined by the siRNA experiment. When AREG was specifically knocked down with siRNA, proliferation was repressed accordingly. In contrast, AREG level was low and siRNA treatment had no effect on proliferation in LTEDaro (Fig. 4). Using proliferation assays, the sensitivity of ExeR cells to ER, MAPK, and EGFR inhibitors (Fig. 5) was shown. Based on the results, it was proposed that exemestane-induced AREG plays an important role in ExeR cell proliferation under the estrogen-restricted condition: exemestane induces AREG expression and AREG subsequently activates EGFR and MAPK, which promote cell proliferation (Fig. 6).

AREG was reported to have oncogenic effects in many cancer cell types and was implicated in drug resistance (10). Originally purified from culture medium of PMA-stimulated MCF-7 cells, AREG was found to bind exclusively to EGFR and to activate related pathways (9). AREG has attracted more attention because EGFR has an important role in tumorigenesis. AREG is an important mediator of G protein–coupled receptor–induced activation of EGFR (14). Hurbin et al. (18) reported the antiapoptotic effect of

AREG through the cooperation of AREG and insulin-like growth factor I (IGF-I) in non–small cell lung cancer cells. O'Reilly et al. (19) reported hypoxia-induced AREG expression in intestinal epithelial cells. AREG may contribute to continued cell proliferation under hypoxia conditions. Castillo et al. (20) reported the existence of an AREG-mediated autocrine loop that contributes to the transformed phenotype of human hepatocellular carcinoma cells. Willmarth and Ethier (21) reported the autocrine and juxtacrine effects of AREG in mammary epithelial cells. AREG is involved in ERα/EGFR cross talk in mammary epithelial cells, and this proposed autocrine loop may be responsible for tamoxifen resistance (22). High serum levels of AREG were reported in non–small cell lung cancer patients with poor response to gefitinib, a specific EGFR inhibitor (23). Besides its role in tumorigenesis, AREG was also reported as an essential mediator of ERα in mammary gland development (24). AREG is highly expressed and apparently has a crucial role in parathyroid hormone–stimulated bone formation (25).

Expression of AREG can be regulated at the transcriptional level and posttranslational level. AREG was shown as an estrogen-regulated gene (6, 7). Johansson et al. (26) showed that AREG expression can be strongly induced by cyclic AMP–elevating reagents such as prostaglandin $E_2$ and folskolin in many cell types including MCF-7. AREG is produced as a membrane-anchored precursor with $NH_2$-terminal glycosylation sites and is secreted through a cleavage process that is dependent on metalloproteinase such as TACE/ADAM17. The different sites of proteinase cleavage lead to the various forms of AREG (10). This essential function of TACE/ADAM17 makes it a potential therapeutic target by limiting the availability of AREG (27).

Exemestane is known to have weak androgenic effects (28). Despite the concerns of bone loss associated with the estrogen depletion effect of aromatase inhibitors in general, the effect of exemestane on bone is still controversial. Two recent studies supported the role of exemestane in bone development and bone protection. In both cases, the authors attributed the possible bone-protective effect to the androgenic structure of exemestane. Using microarray analysis, Miki et al. (29) reported on exemestane treatment in human osteoblast and osteoblast-like cells. They found that exemestane treatment induced many genes related to cell proliferation and genes encoding cytoskeleton proteins (29). Recently, Goss et al. (30) reported that exemestane increased the serum level of procollagen I $NH_2$-terminal propeptide, a marker of bone formation. From the results of a clinical trial on 128 women with early breast cancer, Lønning et al. (31) concluded that exemestane modestly enhanced bone loss. A careful analysis of these reports suggests that the androgenic effect of exemestane may be related to the relatively high dose.

A study of the mechanism of hormone independency has both academic significance and clinical applications. Previous studies were mostly focused on tamoxifen resistance. Because of the increasing use of aromatase inhibitors in breast cancer treatment, a few groups including our lab have initiated research on aromatase inhibitor resistance. Strictly, we are studying "acquired resistance." That means the cells were originally responsive to aromatase inhibitor and gained resistance gradually. The consensus of current knowledge suggests many commonalities between aromatase inhibitor resistance and endocrine resistance in general, as reviewed by Normanno et al. (11), Dowsett et al. (32), and Chen et al. (2). Briefly, the acquired resistance is a progressive process under selective pressure. Cells evolved from hormone dependent into hormone insensitive and finally hormone independent (or described otherwise as hormone therapy responsive to insensitive to resistant). Cross talk between ER pathway and growth factor pathways (such as EGFR, HER2, and IGF-I receptor) are identified as key mechanisms. Activated phosphatidylinositol 3-kinase, AKT, and MAPK pathways are detected as the consequence of the cross talk.

In our present study, we showed that exemestane-induced AREG expression is essential to the proliferation of ExeR cells. By using ICI, we also showed that the induction of AREG expression is ER dependent. ICI is well known as a "pure antiestrogen" with ER-destabilizing ability (15). By using an aromatase assay, we further showed that there is unlikely any direct antagonism of ICI on exemestane-mediated inhibition of aromatase. Because AREG is known as an "estrogen-induced" gene, this effect strongly suggests the "estrogen-like" function of exemestane. Additional experiments are being done in our laboratory to show the estrogen-like activity of exemestane. Hopefully, these studies will reveal more of a detailed mechanism of how exemestane and ER are involved in the induction of estrogen-responsive genes. The current study again points out the importance of the cross talk between ER and EGFR in hormone therapy resistance. Specifically, ER-mediated AREG expression is crucial in mediating exemestane resistance in our ExeR model. To the best of our knowledge, this is the first report of a resistance mechanism that is specific to exemestane. Our current study provides critical molecular information that will help the development of treatment strategies against exemestane resistance.

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