Transcriptional activation of cathepsin D gene expression by growth factors

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ABSTRACT

Insulin-like growth factor-I (IGF-I), transforming growth factor α (TGFα) and epidermal growth factor (EGF) induced cathepsin D gene expression and reporter gene activity in MCF-7 human breast cancer cells transiently transfected with a construct (pCD1) containing a −2576 to −124 cathepsin D gene promoter insert. In contrast, IGF-I, but not TGFα or EGF, induced reporter gene activity in cells cotransfected with wild-type estrogen receptor (ER) expression plasmid and a construct (pCD2) containing estrogen-responsive downstream elements from −208 to −101. Promoter deletion and mutational analysis experiments identified four GC-rich sites and an imperfect palindromic estrogen responsive element required for IGF-I activation of the ER (ligand-independent). Subsequent studies with the mitogen-activated protein kinase (MAPK) inhibitor, PD98059, and a serine118-ER mutant confirmed the role of the MAPK pathway for IGF-I activation of the ER in MCF-7 cells. Thus, growth factor activation of ER can mediate transactivation vs ER/Sp1 binding to GC-rich sites and represents a novel pathway for ligand-independent ER action. The divergent pathways for IGF-I and TGFα/EGF activation of the ER observed in MCF-7 cells contrast with previous data indicating that pathways for growth factor activation of the ER are dependent on the gene and/or gene promoter and on cell context.

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INTRODUCTION

Cathepsin D is a proteolytic enzyme that is normally localized in lysosomes and functions in protein catabolism. Cathepsin D and the higher molecular procathepsin D precursor are expressed in estrogen receptor (ER)-positive MCF-7 breast cancer cells; 17β-estradiol (E2) induces secretion of procathepsin D and cathepsin D in this cell line (Morisset et al. 1986, Biegel & Safe 1990). The human cathepsin D gene contains nine exons and expresses a single 2·2 kb transcript (Augereau et al. 1988, Redecker et al. 1991). Cathepsin D expression is under complex hormonal and mitogenic control that is cell- and tissue-specific. Human breast cancer cells express up to 30-fold more cathepsin D mRNA than normal mammary cells (Rochefort 1990). In MCF-7 cells, E2 induces up to a 10-fold increase in cathepsin mRNA levels that is not inhibited by cycloheximide, thus indicating an increased rate of transcription (Cavailles et al. 1988). In addition, 8-bromo-cAMP, an inducer of intracellular cAMP levels, also induces cathepsin D mRNA in MCF-7 cells (Chalbos et al. 1993).

Cathepsin D is transcribed from five start sites; however, transcriptional activation by estrogens is TATA-dependent (Cavailles et al. 1993). Initial studies of the cathepsin D gene promoter demonstrated that E2 responsiveness was primarily associated with the −252 to −124 region of the promoter, and various sequences within this region were protected in a DNase I footprinting experiment (Cavailles et al. 1991, 1993, Augereau et al. 1994). Deletion analysis of the cathepsin D gene promoter in MCF-7 cells identified three regions at −208 to −161, −145 to −119 and −120 to −101 that were required for ERα activation. The upstream sequence contained a GC-rich Sp1 binding site and an estrogen-responsive element half-site (ERE½) that formed an ER/Sp1
protein–DNA complex in gel mobility shift assays (Krishnan et al. 1994, 1995). E2-induced transactivation at the overlapping GC-rich site (−145 to −135) was dependent on ER/Sp1-DNA interactions that did not require direct ER binding to promoter DNA (Wang et al. 1998). The downstream region (−120 to −101) contained an imperfect palindromic ERE (Wang et al. 1997) and an adjacent E-box motif (Xing & Archer 1998) that are required for E2 responsiveness.

Cavailles and coworkers (1989) previously reported that growth factors induced cathepsin D gene expression, and this study has confirmed that transforming growth factor α (TGFα), epidermal growth factor (EGF) and insulin-like growth factor-I (IGF-I) induced chloramphenicol acetyltransferase (CAT) activity in cells transiently transfected with pCD1 (contains a −2576 to −124 cathepsin D gene promoter insert linked to a CAT reporter gene). Deletion analysis of the cathepsin D gene promoter shows that IGF-I-induced activity is primarily associated with ligand-independent activation of ER and subsequent interaction with E2-responsive elements identified within the E2-responsive proximal region (−208 to −101) of the promoter. In contrast, EGF/TGFα do not induce through activation of these ER-dependent promoter elements, illustrating divergent pathways for IGF-I- and EGF/TGFα-mediated induction of cathepsin D gene expression in breast cancer cells. With the exception of two additional GC-rich upstream sequences in the promoter (−208 to −161), both IGF-I and E2 activate promoter-reporter activity through the same elements, and ligand-independent activation by IGF-I requires phosphorylation of Ser118 of the ER through the mitogen-activated (protein) kinase pathway (MAPK).

### MATERIALS AND METHODS

#### Chemicals and biochemicals

Dulbecco’s modified Eagle’s medium nutrient mixture F-12 Ham (DME F-12) without phenol red, phosphate-buffered saline (PBS), acetyl coenzyme A (CoA), E2, 100 × antibiotic/antimycotic solution, IGF-I, TGFα, and EGF were purchased from Sigma Chemical Company (St Louis, MO, USA). Fetal calf serum (FCS) was obtained from Intergen (Purchase, NY, USA). Minimum Essential Medium (MEM) was purchased from Life Technologies (Grand Island, NY, USA). [14C]Chloramphenicol (53 mCi/mmol) was purchased from NEN Research Projects (Boston, MA, USA). Poly d(I-C), restriction enzymes (HindIII and BamHI, etc.) and T4-polynucleotide kinase were purchased from Boehringer Mannheim (Indianapolis, IN, USA). Wild-type human ER (hER) expression plasmid was kindly provided by Dr Ming-Jer Tsai, Baylor College of Medicine (Houston, TX, USA) and the HEGO-Ser118 mutant ER (mER-Ser118) was kindly provided by Dr DA Lannigan, University of Virginia (Charlottesville, VA, USA) (Joel et al. 1998). Oligonucleotides were synthesized and purchased from either Gene Technologies Laboratory (Texas A & M University, TX, USA), Genosys Biotechnologies, Inc. (Woodlands, TX, USA), or Life Technologies. PD98059 was purchased from Biomol Research Laboratories, Inc. (Plymouth Meeting, PA, USA). Plasmid preparation kits were purchased from Qiagen (Santa Clarita, CA, USA). All other chemicals and biochemicals were the highest quality available from commercial sources.

Table 1 lists the oligonucleotide sequences (sense strand) used in this study to prepare the corresponding plasmids. The numbering is based on +1 as the first nucleotide of translation codon as described (Cavailles et al. 1993, Augereau et al. 1994). The serum response element (SRE) oligonucleotide is derived from the c-fos protooncogene promoter (−325 to −296) (Treisman 1992).

#### Plasmids

DNA upstream of exon 1 of the human cathepsin D gene was isolated from a lambda clone containing a 10 kg EcoRI insert, by partial digestion with EagI and digestion with EcoRI. The 3′ EagI site corresponds to CGGCCG 10 bases upstream of the start codon in exon 1. This fragment was subcloned into a plasmid vector with a CAT reporter gene (to give pCD1) from which it was released as a 5′ EcoRI to 3′ HindIII 2·65 kb insert. Other constructs used in this study were obtained from the corresponding double-stranded synthetic oligonucleotides and were prepared by ligation of appropriate double-stranded oligonucleotides into pB/L/TATA/CAT vector using HindIII and BamHI restriction enzyme sites, as previously described (Krishnan et al. 1994, 1995, Wang et al. 1997, 1998). All ligation products were transformed into DH 5α competent E. coli cells, plasmids were isolated, and correct clonings were confirmed by restriction enzyme mapping and DNA sequencing using a Sequitherm cycle sequencing kit from Epicentre Technologies (Madison, WI, USA). Constructs pCD3 and pCD3m2 are comparable to the wild-type (ER/Sp1-tk-CAT) and mutant (ER/Sp1-tk-CAT) constructs previously described (Krishnan et al. 1994). Plasmid preparation for
transfection utilized alkaline lysis followed by two cesium chloride gradient centrifugations or the Qiagen Plasmid Mega Kit.

**Cell culture maintenance and growth**

MCF-7 and MDA-MB-231 human breast cancer cells were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA), and MCF-7 cells were maintained in MEM with phenol red and supplemented with 10% FCS plus 0.2 × antibiotic/antimycotic solution, 0.035% sodium bicarbonate, 0.011% sodium pyruvate, 0.1% glucose, 0.23% Hepes, and 6 × 10⁻⁷% insulin. MDA-MB-231 cells were maintained in DME F-12 media supplemented with 5% FCS, 0.5 × antibiotic/antimycotic solution and 0.22% sodium bicarbonate. Cells were passaged every three to five days without becoming confluent.

**Transient transfection and CAT assays**

Cells grown under maintenance were trypsinized, seeded in 100-mm petri dishes with 10 ml phenol red-free DME F-12 medium plus 1% charcoal-stripped FCS, and grown until 50–60% confluent. One to three hours prior to transfection, the medium was replaced with 4 ml of the charcoal-stripped DME F-12 medium. Cells were transfected with 1 ml transfection cocktail containing 8 μg test plasmid, 1.5 μg of a β-galactosidase-lacZ plasmid (Invitrogen, Carlsbad, CA, USA), 1.5 μg hER or mER-Ser 118, 5 µl 2 X Hepes buffer saline (HBS) (pH 7.05). Due to overexpression of the constructs, cotransfection with ER is required for ER action, and this has previously been reported in MCF-7 cells for other constructs including those that have single palindromic ERE inserts derived from the frog vitellogenin A2 gene promoter and other plasmids containing promoter inserts from E2-responsive cathepsin D, c-myc, retinoic acid receptor α1, progesterone receptor, Hsp 27, c-fos and pS2 genes (Weisz & Rosales 1990, Cavailles et al. 1991, Savouret et al. 1991, Dubik & Shiou 1992, Cavailles et al. 1993, Augereau et al. 1994, Krishnan et al. 1994, Zacharewski et al. 1994, Rishi et al. 1995, Porter et al. 1996, Sathya et al. 1997). After incubation for 14–16 h at 37 °C in air:CO₂ (95%:5%), cells were washed once with 5 ml PBS and treated with growth

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**Table 1. Oligonucleotide sequences (sense strand) used in this study to prepare the corresponding plasmids**

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Oligonucleotide sequence</th>
</tr>
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<tbody>
<tr>
<td>CD2 (-208 to -101)</td>
<td>5’-AGCTTCCCCCGCCCCCGCCGGCGCTGTGCCGTGCCGGAGG</td>
</tr>
<tr>
<td>CD3 (-208 to -161)</td>
<td>1TGGCCCCGGGAGCCAGGAGCGCCGGGCGCTTCGCCCCGCCCCGCGCA</td>
</tr>
<tr>
<td>CDm1 (-208 to -161)</td>
<td>GCCGGGGCCGGCCGACCTGAAAGGCTGGGTCCG-3’</td>
</tr>
<tr>
<td>CDm2 (-208 to -161)</td>
<td>5’-AGCTTCCCCCCCCCGGCTGAGCTGGGTCCG-3’</td>
</tr>
<tr>
<td>CDm3 (-208 to -161)</td>
<td>GCCGGGGCCGGCCGACCTGAAAGGCTGGGTCCG-3’</td>
</tr>
<tr>
<td>CD4 (-145 to -119)</td>
<td>5’-AGCTTCCCCCGCCGCACGCACTGAGGCTGGGTCCG-3’</td>
</tr>
<tr>
<td>CDm4 (-145 to -119)</td>
<td>GCCGGGGCCGGCCGACCTGAAAGGCTGGGTCCG-3’</td>
</tr>
<tr>
<td>CD5 (-120 to -101)</td>
<td>5’-AGCTTGCGCCACGTGACCCGGG-3’</td>
</tr>
<tr>
<td>CDm5 (-120 to -101)</td>
<td>GCCGGGGCCGGCCGACCTGAAAGGCTGGGTCCG-3’</td>
</tr>
<tr>
<td>CD6 (-145 to -101)</td>
<td>5’-AGCTTCCCCGCCCCGCGCCGACGCACTGACGTCCG-3’</td>
</tr>
<tr>
<td>SRE (-325 to -296)</td>
<td>5’-AGCTTACACAGGTGCTCATATTTTAGGACATCTGAGC-3’</td>
</tr>
</tbody>
</table>

The HindIII and BamHI linker sequences are italicized. The bold letters indicate the imperfect palindromic ERE sequence, Sp1 binding site, or dioxin response element (DRE), and their mutated bases are underlined. The numbers in parentheses indicate the positions within the 5’-promoter region of cathepsin D gene. The numbering is based on +1 as the first nucleotide of translation codon as described (Cavailles et al. 1993, Augereau et al. 1994). The SRE oligonucleotide is derived from the c-fos protooncogene promoter (−325 to −296) (Treisman 1992).
factors and/or PD98059. After 24 to 30 h, cells were harvested by scraping. Cells were lysed in 200 µl 0.25 M Tris–Cl (pH 7.6) and freeze-thawed (3 ×) in liquid nitrogen for 2 min. The cell debris was pelleted and the protein concentrations were measured by the method of Bradford (1976) using bovine serum albumin as standard. An aliquot of cell lysate was brought to 120 µl with 0.25 M Tris–Cl (pH 7.6) and incubated with 1 µl [14C]chloramphenicol (53 mCi/mmol) and 40 µl 4 mM acetyl CoA for 4 h at 37 °C. After vortexing with 700 µl ethyl acetate for 30 s, the mixture was centrifuged at 16 000 g for 1 min at room temperature. A 600 µl aliquot of ethyl acetate was evaporated in vacuo, resuspended in 20 µl ethyl acetate, and separated by thin-layer chromatography using a 95:5 chloroform: methanol solvent as previously described (Wang et al. 1997, 1998). The percentage protein conversion into acetylated chloramphenicol was quantitated using the counts/min obtained from the Betagen Betascope 603 blot analyzer (Intelligent, Mountain View, CA, USA). CAT activity was normalized to β-galactosidase enzyme activity obtained after cotransfection with a β-galactosidase-lacZ plasmid (1-50 µg). Initial studies (see Figs 1, 2, 3A,B) standardized transfections using protein concentrations, whereas results in Figs 3C, 4 and 5 standardized transfections using the cotransfection assay, and both assays gave similar results.

**Northern blot analysis**

Cathepsin D mRNA levels were determined by using a 1.2-kb EcoRI fragment of the human cathepsin D cDNA. β-Tubulin mRNA levels were determined by using a 1.1-kb EcoRI fragment of human β-tubulin cDNA. Total RNA was isolated by the RNAzol B reagent (TelTest, Friendswood, TX, USA). Total RNA (20 µg) was separated in a 1% sodium phosphate/2 mM EDTA gel in 20 mM sodium phosphate/2 mM EDTA, transferred onto a nylon membrane by capillary action, and bound to the membrane by UV crosslinking. cDNAs were labeled with [α-32P]dCTP by using a random primer DNA labeling system (Boehringer Mannheim), and the radiolabeled cDNA probe was added at 2 × 10^6 c.p.m./ml hybridization solution (5 × SSPE (1 × SSPE is 0.15 M NaCl, 10 mM NaH2PO4, 1 mM EDTA, pH 7.4), 1% sodium dodecyl sulfate (SDS), 10% dextran sulfate, 5 × Denhardt’s solution). Hybridizations were performed in roller bottles at 65 °C for 20 h. Nonspecifically-bound probe was removed by 15 min (2 ×) and 20 min (1 ×) washes at 20 °C in 1 × SSPE, two 30-min washes at 65 °C in 0.1 × SSPE-1% SDS, and one 15-min wash at 20 °C in 1 × SSPE. Membranes were stripped of probe by boiling for 20 min in 0.1 × SSPE-0.5% SDS. Bands were scanned with Adobe Photoshop 3.0 (Mountain View, CA, USA) and quantitated with ZERO-Dscan (Scanalytics, Billerica, MA, USA).

**Statistics**

Results are expressed as means ± s.e. for at least three independent (replicate) experiments for each treatment group. Statistical significance was determined by ANOVA and Student’s t-test and the levels of probability are noted for each experiment.

**RESULTS**

Previous studies have demonstrated that growth factors induce cathepsin D gene expression in MCF-7 cells (Cavaillles et al. 1989), and the results in Fig. 1 demonstrate that IGF-I significantly induces cathepsin D mRNA levels and also causes a 2- to 3-fold increase in CAT activity in MCF-7 cells transiently transfected with pCD1 containing a −2576 to −124 cathepsin D gene promoter insert. In addition, both EGF and TGFβ induce CAT activity in MCF-7 cells transfected with pCD1. In contrast, only IGF-I significantly induced CAT activity (2-9-fold) in cells transfected with pCD2 (Fig. 1) suggesting that the −108 to −101 region of the cathepsin D gene promoter was associated with IGF-I-responsiveness, whereas upstream elements are required for TGFβ/EGF action. MCF-7 cells express the IGF-I and EGF receptors (Davidson et al. 1987, Cullen et al. 1990), however, it is possible that differences between IGF-I and EGF may be due to decreased kinase activation by EGF. Results in Fig. 1D show that IGF-I and EGF activate the pSRE construct indicating that both growth factors induce kinase-dependent activation pathways in MCF-7 cells. Interestingly, IGF-I and E2 activation of constructs derived from the −208 to −101 region of the promoter all required cotransfection with ERα for expression plasmid, whereas growth factor activation of pSRE did not require further expression of growth factor receptors.

Three E2-responsive regions of the cathepsin D gene promoter have been characterized at −208 to −161, −145 to −119, and −120 to −101 (Krishnan et al. 1994, 1995, Wang et al. 1997, 1998, Xing & Archer 1998), and these sequences have been inserted upstream from a CAT gene sequence in pBL/TATA/CAT2 to give pCD3, pCD4 and pCD5. Results illustrated in Fig. 2 show that IGF-I, but not TGFβ or EGF, induced CAT activity (3-3 fold induction for IGF-I) in cells...
transiently transfected with pCD3. Similar results were obtained with MCF-7 cells transfected with pCD4 and pCD5 in which treatment with IGF-I induced a 2.0- and 2.6-fold increase in CAT activity, respectively, whereas induction responses were not observed for EGF and TGFα. Induction responses by IGF-I required cotransfection with wild-type ER expression plasmid (data not shown).

Previous studies have demonstrated that elements required for ERα-dependent activation of pCD3, pCD4 and pCD5 include the GC-rich sites in the former two constructs and an imperfect palindromic ERE overlapping an E-box, respectively (Krishnan et al. 1994, 1995, Wang et al. 1997, 1998, Xing & Archer 1998). Therefore, the role of these sites in IGF-I-mediated transactivation was investigated using mutant constructs pCD3m, pCD4m and pCD5m that are not E2-responsive. The results (Fig. 3) clearly demonstrate that in cells transiently transfected with the mutant pCD4m and pCD5m constructs, IGF-I did not induce reporter gene expression, whereas induction was observed using the corresponding wild-type constructs. The −208 to −161 region of the cathepsin D gene promoter contains an E2-responsive Sp1(N)23ERE½ motif and mutation of either the Sp1 binding site or ERE½ results in loss of E2 responsiveness (Krishnan et al. 1994, 1995). However, the results in Fig. 3C show that IGF-I induced CAT activity using the pCD3m1 construct mutated in the GC-rich site that forms part of the Sp1(N)23ERE½ motif. The −208 to −161 region also contains two additional GC-rich sites at −206 to −201 and −167 to −162, and pCD3m2 and pCD3m3 contain

FIGURE 1. Growth factor-mediated induction of cathepsin D. (A) E2- and IGF-I-mediated cathepsin D (CATH-D) gene expression. Total RNA was isolated from MCF-7 cells treated with dimethyl sulfoxide (DMSO) (control), 10 nM E2, or 10 nM IGF-I for 24 h. The Northern blot was visualized by autoradiography and quantitated as described in Materials and Methods. Cathepsin D mRNA levels were normalized to β-tubulin mRNA for each treatment group, and relative cathepsin D mRNA levels were 100 ± 19 (control), 264 ± 12 (E2), and 232 ± 6 (IGF-I). There was a significant increase (P<0.05) in cathepsin D mRNA levels in E2- or IGF-I-treated cells. (B) Transfection with pCD1. MCF-7 cells were cotransfected with pCD1 and wild-type ER, treated with DMSO, 10 nM IGF-I, EGF or TGFα, and CAT activity was determined as described in Materials and Methods. Significant induction (P<0.05) was observed only for IGF-I. (C) Transfection with pCD2. This experiment with pCD2 was carried out as described in (B). Significant induction (P<0.05) was observed only for IGF-I. (D) IGF-I and EGF induction with pSRE. MCF-7 cells were transfected with pSRE (no ER expression plasmid), treated with 10 nM IGF-I or EGF, and CAT activity determined as described in (B). Both growth factors significantly induced (P<0.05) CAT activity. Results are presented as means ± s.e. for three replicate experiments for each treatment group.
a second mutation and mutations at all three GC-rich sites respectively. The results show that IGF-I induces reporter gene activity in MCF-7 cells transfected with pCD3 (A), pCD4 (B) or pCD5 (C), and wild-type ER expression plasmid and treated with 10 nM IGF-I, EGF or TGFα. CAT activity was determined as described in Materials and Methods. IGF-I significantly (P<0.05) induced CAT activity with all constructs, whereas EGF and TGFα were inactive. Results are presented as means ± s.e. for three replicate experiments for each treatment group.

![Image](image.png)

**FIGURE 2.** Growth factor induction after transfection with pCD3, pCD4 and pCD5. MCF-7 cells were transfected with pCD3 (A), pCD4 (B) or pCD5 (C), and wild-type ER expression plasmid and treated with 10 nM IGF-I, EGF or TGFα. CAT activity was determined as described in Materials and Methods. IGF-I significantly (P<0.05) induced CAT activity with all constructs, whereas EGF and TGFα were inactive. Results are presented as means ± s.e. for three replicate experiments for each treatment group.

It has been reported that IGF-I-induced activation of ER was dependent on the MAPK pathway (Kato et al. 1995); therefore, the effects of the MAPK inhibitor PD98059 were determined in MCF-7 cells transiently transfected with pCD3,
pCD4 or pCD5 and treated with IGF-I (Fig. 4). The results show that IGF-I-induced CAT activity is significantly inhibited by PD98059 in MCF-7 cells transfected with all three constructs, suggesting a role for the MAPK pathway in IGF-I-mediated ER activation of cathepsin D.

Previous studies have demonstrated that MAPK-dependent phosphorylation of Ser118 plays an important role in growth factor activation of ER (Kato et al. 1995); therefore, we investigated the role of Ser118 using a mutant ER-Ser118 expression plasmid in ER-negative MDA-MB-231 breast cancer cells transfected with E2-responsive constructs derived from the cathepsin D gene promoter. Preliminary studies showed that pCD4 and pCD5 were only weakly inducible by IGF-I in MDA-MB-231 cells, and therefore a combined construct (pCD6), previously shown to be E2-responsive (Wang et al. 1997), was utilized for this study. IGF-I induced reporter gene activity in MDA-MB-231 cells transiently cotransfected with pCD3 or pCD6 and wild-type ER, whereas no induction was observed when cells were cotransfected with mutant ER-SER118 expression plasmid (Fig. 5). The comparative activation of pCD3 in MCF-7 cells transfected with wild-type ER and ER-Ser118 gave similar results except that some induction was observed, and this may be due to endogenous expression of wild-type ER in this cell line. These results suggest the important role of Ser118 phosphorylation of ER by IGF-I for activation of cathepsin D by ER (ligand-independent) in breast cancer cells.

**DISCUSSION**


Previous studies have reported that IGF-I and EGF induce cathepsin D gene expression in ER-positive breast cancer cells (Cavailles et al. 1989); this was also noted in this study and comparable induction of CAT activity was observed in MCF-7 cells transiently transfected with pCD1
and a construct containing a serum response element promoter (pSRE) (Fig. 1). Thus, elements required for responsiveness to IGF-I, EGF and TGFβ are present in the −2576 to −124 region of the cathepsin D gene promoter, and both IGF-I and EGF activate through a serum response element indicating that functional growth factor receptors are expressed in these cells, as previously described (Furlanetto & DiCarlo 1984, Davidson et al. 1987, Cullen et al. 1990, Freiss et al. 1990). E2-responsiveness of the cathepsin D gene is primarily located in the downstream proximal region of the promoter (Cavailles et al. 1991, Augereau et al. 1994), and at least three E2-responsive motifs have been identified (Krishnan et al. 1994, 1995, Wang et al. 1997, 1998, Xing & Archer 1998). The role of the ER in mediating growth factor signaling was investigated using both wild-type and mutant constructs containing cathepsin D gene promoter elements derived from the downstream E2-responsive motifs, ES-1, ES-2 and ES-3 (Fig. 6). Results (see Figs 1 and 2) clearly show that IGF-I, but not EGF or TGFβ, activates gene expression through these E2-responsive elements, and this distinctly differentiates between IGF-I and EGF/TGFβ activation of the cathepsin D gene. Similar differences have also been observed in MCF-7 cells transfected with a construct containing a perfect palindromic ERE promoter; IGF-I and E2 induced reporter gene activity (Hafner et al. 1996, Lee et al. 1997), whereas EGF was inactive (Hafner et al. 1996). Previous studies reported a common pathway for growth factor-ER signaling in ER-negative cells and in ER-positive BG-1 ovarian adenocarcinoma cells (Nelson et al. 1991, Ignar-Trowbridge et al. 1993, 1996, Kato et al. 1995, El-Tanani & Green 1997); however, results of this study show divergent pathways for IGF-I-ER and TGFβ/ER action, demonstrating that pathways for growth factor activation of the ER are cell context- and possibly promoter-dependent. Current studies are focused on characterizing upstream elements in the cathepsin D gene promoter associated with EGF/TGFβ-responsiveness in breast cancer cells. Confirmation of IGF-I action on E2-responsive motifs within the −201 to −101 region of the cathepsin D gene promoter was determined using
mutant constructs (Fig. 3), the MAPK inhibitor PD98059 (Fig. 4), and mER-Ser\textsuperscript{118} (Fig. 5). The results were consistent with IGF-I activation of ER through the ras-MAPK pathway and confirmed that phosphorylation of SER\textsuperscript{118} was important from through the ras-MAPK pathway and con & Sirbasku 1988, Wilding et al. et al. in MCF-7 cells (Furlanetto & DiCarlo 1984, Bates Previous studies indicate that TGF growth factor activation of the ER is complex and is activates ER (ligand-independent)-mediated induc- results of this study demonstrate that only IGF-I ligand-dependent activation of pCD3 by E2 is more until all three Sp1 binding sites are mutated. Thus, ligand-independent activation of ER by IGF-I. One surprising result was obtained with mutants derived from the – 208 to – 161 region of the cathepsin D gene promoter. Mutation of the GC-rich site (– 199 to – 194) that forms part of the Sp1(N\textsubscript{23})ERE\textsubscript{½} motif, to give pCD3m1 (Fig. 3C) did not result in loss of IGF-I responsiveness, whereas this construct is not E2-responsive in MCF-7 cells (Krishnan et al. 1994). However, this region of the promoter contains two additional GC-rich sites and IGF-I mediated transactivation of mutant pCD3 constructs is not lost until all three Sp1 binding sites are mutated. Thus, ligand-independent activation of ER by IGF-I may involve the three GC-rich sites in pCD3, whereas ligand-dependent activation of pCD3 by E2 is more selective and is activated through only one of these sites (Krishnan et al. 1994).

These studies demonstrate that the pattern of growth factor activation of the ER is complex and is dependent on the gene promoter and cell context. Previous studies indicate that TGF\alpha, EGF and IGF-I induce cell proliferation and gene expression in MCF-7 cells (Furlanetto & DiCarlo 1984, Bates et al. 1988, Dickson & Lippman 1988, Karey & Sirbasku 1988, Wilding et al. 1988, Cavailles et al. 1989, Freiss et al. 1990, Katzenellenbogen & Norman 1990, Dickson & Lippman 1991, Fernandez et al. 1994, Westley & May 1994, Dickson & Lippman 1995, Smith 1998); however, results of this study demonstrate that only IGF-I activates ER (ligand-independent)-mediated induc- tion of reporter gene activity through multiple E2-responsive elements in the proximal promoter region of the cathepsin D gene. These data indicate divergent pathways for IGF-I and TGF\alpha/EGF in breast cancer cells. Current studies in this labora- tory are focused on identifying specific factors responsible for cell-specific differences in growth factor activation of the ER.

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