Regulation of estrogen receptor-α expression in MCF-7 cells by taxol

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Abstract

Results presented in this study demonstrate that treatment of MCF-7 cells with taxol resulted in induction of estrogen receptor-α (ERα) gene transcription with a subsequent increase in ERα mRNA; this effect was promoter specific since taxol did not affect total transcription in MCF-7 cells and lacked an effect on transcription of the human acidic ribosomal phosphoprotein PO, progesterone receptor, and pS2 genes. In contrast to the increase in transcription of the ERα gene, taxol inhibited translation of the ERα mRNA. This effect is also transcript specific since taxol did not alter total protein synthesis and did not affect the concentration of progesterone receptor protein in the cell. The overall result of taxol treatment was to decrease the concentration of ERα protein in the MCF-7 cells. Evidence is presented that the effects of taxol on ERα gene transcription may be mediated through the induction of p53.


Introduction

The presence of estrogen receptor-α (ERα) and progesterone receptor (PR) in breast tumors is used to predict those patients who would benefit from hormonal therapy (Allegra & Lippman 1980, DeSombre & Jensen 1980, Osborne et al. 1980, Paridaens et al. 1980). Unfortunately a high percentage of receptor-positive tumors become resistant to this therapy. The mechanism responsible for the loss of estrogen responsiveness in human breast cancer remains unclear and it is the reason for the broad interest in studying the regulation of ERα gene expression. The results from these studies suggested that regulation of the steady-state level of ERα in breast cancer cells is a complex phenomenon that includes transcriptional and post-transcriptional mechanisms (Saceda et al. 1988, 1991, 1996, Lee et al. 1989, Cho et al. 1991, Garcia-Morales et al. 1994, Martin et al. 1995, Stoica et al. 1997, 2000a,b) which are tightly regulated by a variety of agents including estradiol (Saceda et al. 1988, Cho et al. 1991), phorbol esters (Lee et al. 1989, Saceda et al. 1991, Martin et al. 1995), growth factors (Saceda et al. 1996, Stoica et al. 1997, 2000a,b), and heavy metals (Garcia-Morales et al. 1994). In this study, the effects of taxol on ERα expression in MCF-7 cells were investigated. Taxol, a diterpenoid isolated from the Pacific yew Taxus brevifolia (Wani et al. 1971, Adams et al. 1993), has been used extensively as an anticancer drug (Long 1994, Crown & O’Leary 2000, Mukherjee et al. 2001). It is effective in several types of cancer, particularly in some hormone-related cancers such as breast carcinoma (Fornier et al. 2000). Taxol has multiple biological effects, including inhibition of cell division (Milas et al. 1995, Milross et al. 1996), inhibition of fibroblast migration (Joseph et al. 1989), changes in cell morphology (Pletjushkina et al. 1994), and alteration of the expression of genes, such as the tumor suppressor gene p53 (Tishler et al. 1995) and tumor necrosis factor (TNF) (Burkhart et al. 1994). Some of the effects of taxol appear to be related to its influence on the tubulin–microtubule system (Schiff & Horwitz 1980). Taxol promotes assembly of microtubules and stabilizes them against depolymerizing agents. Taxol has also been reported to have effects that are mediated by mechanisms other than its interaction with microtubules (Moos & Fitzpatrick 1998, Sravastava et al. 1998, Stein 1999, Wang et al. 1999). In chromaffin cells, taxol affects catecholamine secretion. Following exposure to taxol, there is an initial increase in catecholamine secretion that is mediated by an increase in intracellular Ca2+ followed by an inhibition in catecholamine secretion that is mediated by its effect on microtubules (Thuret-Carnahan et al. 1985, Gomez et al. 2000). In the present study, the effects of taxol on ERα expression in MCF-7 cells...
human breast carcinoma cells were investigated and we have also demonstrated a dual effect of the drug on ERα expression. Treatment of cells with taxol resulted in an induction of ERα gene transcription that was accompanied by an increase in ERα mRNA. In contrast to its effect on transcription, taxol inhibited translation of the ERα mRNA. The overall result of taxol treatment was a decrease in the steady-state level of ERα protein. The effects of taxol appear to be specific for ERα expression since transcription and protein synthesis were not affected by treatment with the drug. Evidence is also presented suggesting that the increase in ERα transcription may be mediated through the induction of p53.

Materials and Methods

Tissue culture

Monolayer cultures of MCF-7 human breast cancer cells were grown in improved minimal essential medium (IMEM) supplemented with 5% (v/v) fetal calf serum (FCS). When the cells were 80% confluent, the medium was replaced with phenol red-free IMEM containing 5% charcoal-treated calf serum (CCS) (Berthois et al. 1986). The CCS was pretreated with sulfatase and dextran-coated charcoal to remove endogenous steroids. After 2 days in these conditions, taxol (ICN pharmaceuticals, Costa Mesa, CA, USA), cycloheximide (Sigma), or both were added for proper processing of the 3' end of the fusion construct. The vector also contains a neomycin resistance cassette driven by the SV40 early promoter. The SV40 poly A sequence is included for proper processing of the 3' end of the fusion construct.

Plasmids

The clones for ERα, pOR300 (Saceda et al. 1988) and Q7 (Garcia-Morales et al. 1994), for the PR (Saceda et al. 1988) and pPR250 (Garcia-Morales et al. 1994), for pS2 (Saceda et al. 1988), and for the human acidic ribosomal phosphoprotein protein PO (Saceda et al. 1988) and p36B4 (Saceda et al. 1988) have been described previously. The riboprobe for p53 is an NcoI fragment subcloned into pGEM 7z (kindly provided by Dr Daniel Eliahu). The wild-type p53 expression vector pC53-SN3 was a kind gift from Dr Bert Vogelstein (Baker et al. 1990, Kern et al. 1992). This vector expresses wild-type p53 under the control of the cytomegalovirus (CMV) promoter. The p53-EGFP chimera expression vector p53-EGFP from Clontech Laboratories Inc. (CA, USA) contains a p53-EGFP expression cassette driven by the immediate-early CMV promoter. The SV40 poly A sequence is included for proper processing of the 3' end of the fusion construct.

Western blot

Treated or untreated MCF-7 cells were washed twice with phosphate-buffered saline (PBS). Cells were scraped and centrifuged at 1000 g for 5 min. The cellular pellet was resuspended in RIPA buffer (150 mM NaCl, 1% NP-40, 0.5% deoxycholate, 0.1% SDS, and 50 mM Tris (pH 8)) and protease and phosphatase inhibitors were added. Cells were kept on ice for 30 min and centrifuged at 16 000 g for 15 min at 4 °C. The cellular pellet was discarded, and the protein content of the cell extract (supernatant) was determined using the Bradford method (Bio-Rad, Richmond, CA, USA). ER expression was determined by Western blot using the monoclonal antibody anti-ERα (D-12) (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) followed by enhanced chemiluminescence (Amersham International, Amersham, Bucks, UK) to develop protein bands.

Measurement of cellular ERα mRNA

Total cellular RNA was extracted from MCF-7 cells by the method of Chomczynski & Sacchi (1987). The amount of ERα mRNA was determined by RNase protection analysis. In summary, homogeneously 32P-labeled antisense molecules (cRNA) were synthesized in vitro from pOR300 (ERα riboprobe) and p36B4 using T7 polymerase. Total RNA (60 µg) was hybridized for 12–16 h to the radiolabeled cRNA. After a 30-min digestion at 25 °C with RNase A, 32P-labeled cRNA probes protected from RNase digestion were resolved by electrophoresis on 6% polyacrylamide gels. The bands
were visualized by autoradiography and quantified by optical densitometry. The amount of ER mRNA was normalized to the amount of the internal control 36B4, which is the cDNA of the human acidic ribosomal phosphoprotein PO.

Isolation of nuclei

Nuclei from MCF-7 cells were isolated after treatment by a method described previously (Saceda et al. 1988). Briefly, the cells were harvested in 5 ml PBS and pelleted by gentle centrifugation at 4 °C. The pellet was resuspended in lysis buffer (10 mM Tris, pH 7-5, 10 mM NaCl, 3 mM MgCl₂, and 0-5% NP-40). After 5 min on ice, the cells were centrifuged at 500 g for 5 min. The pellet was again resuspended in 4 ml lysis buffer and centrifuged as described above. The pellet from the second centrifugation was resuspended in 500 µl nuclei storage buffer (20 mM HEPES, 75 mM NaCl, 0-125 mM phenylmethylsulfonylfluoride, 0-85 mM dithiothreitol (DTT), 0-125 mM phenylmethylsulfonylfluoride, and 50% glycerol). Nuclei were stored at -70 °C until the transcription elongation assay was performed.

Transcription elongation assay

The nuclear transcription run-on assay was performed as previously described (Saceda et al. 1988). Isolated nuclei were incubated with 32P-UTP and unlabeled ATP, CTP, and GTP. Radio-labeled RNA transcripts were isolated and hybridized to an excess of denatured plasmid DNA immobilized on a nitrocellulose membrane. Denatured plasmids used for detection of specific transcripts were exon 1 of ERα (Q7), ribosomal protein PO, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), pS2, and PR. Autoradiographs were analyzed by densitometry and the background was subtracted. The results were normalized to the transcriptional level of the ribosomal protein PO gene.

Total transcription

Nuclei from MCF-7 cells were isolated after incubation with taxol at different intervals as described above. The nuclear transcription assay was performed as described above except that 5 µCi 3H-UTP were used instead of the 32P-UTP. The transcription reaction was stopped by the addition of 10% trichloroacetic acid (TCA) containing 15 mM sodium pyrophosphate. After incubation on ice for 60 min, the samples were centrifuged at 15 000 g for 20 s. The pellets were washed three times with 5% TCA containing 15 mM sodium pyrophosphate at 4 °C. The pellets were hydrolyzed with 0-4 M perchloric acid at 70 °C for 20 min, followed by neutralization with 0-4 M NaOH. The total radioactivity released was counted in a liquid scintillation counter.

Transfections

Stably transfected p53-EGFP-expressing MCF-7 cells were made as follows. Cells were plated onto 6 cm Falcon plates at 6 × 10⁵ cells/plate in IMEM containing 5% fetal bovine serum (FBS). After overnight incubation at 37 °C and 5% CO₂, cells were washed three times with PBS and overlaid with 3 ml fresh IMEM containing 5% FBS. A mixture of DNA/Superfect reagent (Quiagen Inc., Valencia, CA, USA) was prepared by mixing 2 ng p53-EGFP DNA with 5 µl Superfect and serum-free IMEM up to 600 µl. This mixture was incubated for 10 min at room temperature to allow DNA/Superfect complex formation. The 600 µl DNA/Superfect mixture was added to the 6 cm Falcon plate containing the cells in 3 ml IMEM. After 3-h incubation at 37 °C and 5% CO₂, cells were washed three times with PBS and the selection medium containing Geneticin (G-418) was added. The ERα–128–chloramphenicol acetyl transferase (CAT) vector was constructed using the genomic clone for the ER Q7 (Saceda et al. 1988). The Er promoter from −128 to +1 nucleotide was generated from Q7 by PCR using primers which also contained a HindIII or XbaI restriction site (AAGCTTGTGTTTGGCTGAG and TCTAGAAGCTCCTGGGCCTCCC). The PCR product was purified by low melting agarose gel electrophoresis and subcloned into PCR II TA cloning vector (Invitrogen Corp., Carlsbad, CA, USA). The cloning vector containing the insert was then digested with HindIII and XbaI and the DNA fragment was purified by low melting point agarose gel electrophoresis. The purified fragment was subcloned into the polylinker region of pCAT enhancer (Promega, Madison, WI, USA) resulting in ERα–128–CAT. The ERα–128–CAT vector was sequenced using the Sequenase kit (USB, Cleveland, OH, USA). Transient transfections of MCF-7 cells were performed as described above for stable transfectants but without antibiotic selection and the DNA/Superfect mixture was prepared by mixing 5 ng plasmid and 12.5 µl Superfect.

DNA electrophoretic mobility shift assay

MCF-7 cells were grown and treated as described above. Nuclear extracts from control and treated cells were isolated following a protocol described previously (Saceda et al. 1996). For the DNA mobility shift assay, four different 32P-labeled oligonucleotides covering the region of the ER gene promoter from −128 to +1 bp were incubated with nuclear extracts from MCF-7 cells for 20 min at room temperature in a buffer containing 10 mM Tris (pH 7.5), 100 mM KCl, 1 mM DTT, 1 mM EDTA, and 6% glycerol. One oligonucleotide, referred to as EP-3, contained the sequence CCTCCAGCACCT TTGTAATGCAATCGAGCTCGGG. Samples were loaded onto a 6% polyacrylamide gel in 1 × TBE.
(50 mM Tris–borate (pH 8.3), and 0.5 mM EDTA) and run at 20 mA per gel at 4 °C. The gel was dried and autoradiography was performed.

Results

Effect of taxol treatment on the concentration of ERα protein

To determine whether taxol alters the expression of ERα, MCF-7 cells were treated with various concentrations of the drug for 24 h. To measure the amount of ERα protein, an enzyme immunoassay was performed. The data in Fig. 1A demonstrate that treatment with taxol at concentrations from 0.35 to 3.5 µM resulted in a decline in total ERα protein by approximately 40%. The concentration of ERα protein declined from 285 fmol/mg protein in control cells to approximately 171 fmol/mg protein in treated cells. To define the time-course of this effect, the amount of ERα protein was determined following treatment with 0.35 µM taxol. The data in Fig. 1B show that treatment with 0.35 µM taxol resulted in a decline in total receptor protein by approximately 40% in 3 h; the amount of ERα protein remained suppressed for as long as 24 h. These data demonstrate a taxol-mediated decrease in ERα protein expression in MCF-7 cells. The decrease in ERα protein was also demonstrated by Western blot in total cellular extracts (Fig. 1C) and in membrane extracts (Fig. 1D).

Effect of taxol treatment on the steady-state level of ERα mRNA

To determine whether the reduction in ERα protein paralleled a reduction in the steady-state level of ERα mRNA, MCF-7 cells were treated with 0.35 µM taxol for various times. The amount of ERα mRNA was measured using an RNase protection assay. In these experiments, changes in ERα mRNA were quantified by scanning densitometry and normalized to the amount of ribosomal protein PO mRNA which is constitutively expressed in the presence of taxol (Fig. 2A). In this study, treatment with 0.35 µM taxol resulted in an approximately 75% increase in the amount of ERα mRNA by 6 h. The amount of ERα mRNA remained elevated for at least 24 h. In contrast to the decrease in ERα protein, taxol induced an increase in ERα mRNA, suggesting that the

Figure 1 Effect of taxol treatment on the concentration of ERα protein. MCF-7 cells were grown in IMEM supplemented with 5% FCS. When the cells were 80% confluent, the medium was changed to phenol red-free IMEM and 5% CCS. Cells were grown in this media for 2–3 days and then treated for 24 h with taxol (0.35–3.5 µM). Following treatment, the concentration of ERα protein was determined by an enzyme immunoassay as described in Materials and Methods. (A) Effect of taxol treatment on total ERα protein levels. Values are the means ± s.e.m. of three separate experiments (n = 9). *P < 0.05. (B) Time-course of the effect of taxol on the concentration of ERα protein, *P < 0.05. Effect of taxol treatment on (C) total ERα protein levels and (D) plasma membrane-associated ERα protein determined by Western blot. Lane 1, control MCF-7 cells; lane 2, MCF-7 cells treated with taxol (0.35 µM) for 24 h.
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The means of PO mRNA. The values are presented as percent of control and are densitometry and normalized to the amount of ribosomal protein mRNA. The amount of ER

Methods. (A) Effect of taxol on the steady-state level of ER

transcription was performed as described in Materials and Methods. For transcription studies, nuclei were isolated, and elongation of transcription was performed as described in Materials and Methods. (A) Effect of taxol on the steady-state level of ERα mRNA. The amount of ERα mRNA was determined by scanning densitometry and normalized to the amount of ribosomal protein PO mRNA. The values are presented as percent of control and are the means ± S.E.M. of three separate experiments. *P < 0.05. (B) Effect of taxol (Tax) on ERα gene transcription. The levels of ERα, PR, and pS2 gene transcription were normalized to the level of ribosomal protein PO transcription. The results are presented as percent of transcription of untreated cells. Values are the means ± S.E.M. (n = 4). *P < 0.05. (Inset) Representative nuclear run-on. Line 1, ERα; line 2, PO; line 3, GAPDH; line 4, pS2; line 5, PR.

drug regulates the amount of ERα protein through either a translational or a post-translational mechanism.

Effect of taxol treatment on the level of ERα gene transcription

To determine the effect of the drug on ERα gene transcription, transcription run-on assays were performed with nuclei isolated from MCF-7 cells treated with 0.35 μM taxol. Newly synthesized transcripts were hybridized to probes immobilized on nitrocellulose blots. The level of transcription was determined by autoradiography and quantified by scanning densitometry. Ribosomal protein PO transcription was used as an internal control and the relative changes in ERα transcripts were normalized to the signal obtained for the ribosomal protein. The data in Fig. 2B show a 2.5-fold increase in ERα gene transcription by 1 h after treatment with taxol. Transcription remained high for at least 6 h. As a control, the transcription of the PR and pS2 genes were analyzed. Data in Fig. 2B show that transcription of both genes was not significantly affected by taxol treatment. To rule out the possibility that the effect on ERα gene transcription was a consequence of a general increase in gene transcription, total transcription was determined in cells treated with 0.35 μM taxol for 6 or 24 h. Total transcription was determined as the incorporation of [3H]-uridine per cell. Taxol had no significant effect on total transcription (data not shown). These results demonstrated that the increase in ERα gene transcription induced by taxol was gene specific and not a consequence of a general increase in total transcription. These data suggest that the increase observed in total ERα mRNA after taxol treatment is due to a specific increase in ERα gene transcription.

Effect of taxol treatment on ERα protein half-life

To determine if the decrease in ERα protein observed after taxol treatment was due to a decrease in the stability of the ERα protein, the half-life of the ERα protein was measured following treatment with the drug. To achieve this goal, MCF-7 cells were treated with 0.35 μM taxol for 6 h, and then treated with 10 μg/ml cycloheximide to block protein biosynthesis. ERα protein content was determined at different times after addition of cycloheximide. Data shown in Fig. 3 demonstrate that there was no difference in the ERα half-life after treatment with taxol. The half-life in control and treated cells was approximately 3 h. These results suggested that the decrease in ERα protein observed after taxol treatment was not due to a decrease in ERα half-life, but may be due to a general effect of the drug on protein biosynthesis or to a specific effect on ERα protein synthesis. To distinguish between these possibilities, the incorporation of [3H]-amino acids into proteins was measured in cells treated with 0.35 μM taxol. These data demonstrate that taxol had no significant effect on protein synthesis (data not shown), suggesting that the effect of taxol on ERα protein is specific and not due to a non-specific decrease in total protein biosynthesis.

Determination of ERα promoter region responsible for taxol regulation

To identify the region of the ERα promoter responsible for the taxol-induced increase in transcription, transient transfection assays were conducted using deletion mutants.
of the ERα promoter from −3200 to −40 bp. These studies indicated that the effects of taxol were mediated by the proximal 128 bp in promoter A (data not shown). Promoter A from −128 to +1 bp was then subcloned upstream of a CAT reporter gene in a construct that also contained an SV40 enhancer. Data presented in Fig. 4A showed a tenfold increase in CAT activity in MCF-7 cells treated with taxol. Interestingly, the same reporter was induced by transient cotransfection with the wild-type p53 gene. To further characterize the region of promoter A involved in the regulation of transcription by taxol, four oligonucleotides, which covered the entire 128 bp region of promoter A, were synthesized and named EP-1 to EP-4. To test the ability of nuclear extracts from taxol-treated cells to bind to the different regions of the ERα promoter, DNA electrophoretic mobility shift assays were performed with nuclear extracts isolated from cells treated with 0.35 µM taxol for either 1 or 6 h. The protein-bound probes were separated from free probe by electrophoresis on native polyacrylamide gels. The results of the mobility shift assays showed no detectable difference in the migration patterns of probes EP-1, EP-2, and EP-4 when the probes were incubated with nuclear extracts isolated from either control or treated cells (data not shown). However, with the EP-3 oligonucleotide, a new band appeared in the cells treated with taxol (Fig. 4B). The intensity of the band increased along with the time of treatment and was competed with an excess of cold EP-3, suggesting the formation of a specific taxol-induced complex with EP-3. However, an excess of cold EP-2 and EP-1 were unable to compete the specific binding to EP-3 (Fig. 4C). Antibodies against p53 also blocked the formation of the taxol-induced complex, providing additional evidence for a role of p53 in the regulation of ERα gene transcription.

To further explore whether p53 may be involved in taxol regulation of ERα gene transcription, the effects of taxol treatment on p53 levels and activity were determined. First, the amount of p53 mRNA was measured after taxol treatment. As shown in Fig. 5A, taxol treatment induced an increase in p53 mRNA in MCF-7 cells. Furthermore, taxol treatment induced an increase in p53 protein in MCF-7 cells stably transfected with a p53-EGFP chimera (Fig. 5B). As a positive control for p53 induction, MCF-7 cells transfected with the chimera were treated with trichostatin A and daunomycin (data not shown). These data suggest that the taxol-induced increase in ERα transcription may be mediated by the induction of p53.

Discussion

The results presented herein demonstrate two different effects of taxol on ERα expression. Treatment of MCF-7 cells with concentrations of taxol from 0.35 to 3.5 µM resulted in a rapid decline in total ERα protein. This decrease remained constant for at least 24 h. Interestingly, the overall decrease in ERα protein was not paralleled by a decrease in ERα mRNA; on the contrary, a significant increase in ERα mRNA was observed after taxol treatment. The increase in ERα mRNA was due to a transcriptional effect as demonstrated by nuclear run-on assays. The decrease in ERα protein concentration, even in the presence of an increase in ERα mRNA, was not due to a decrease in the ERα protein half-life, suggesting that taxol blocked the biosynthesis of ERα. The effects of taxol on the transcription and translation of ERα were not due to non-specific effects on total transcription or protein biosynthesis as demonstrated by the lack of an effect of taxol on the incorporation of 3H-uridine and 3H-amino acid into macromolecules. In addition, taxol had no effect on the transcription of the PR, pS2, and ribosomal protein PO genes. Taken together, these data demonstrate multiple effects of taxol on ERα expression: (1) a specific increase in the transcription of the ERα gene and (2) a specific decrease in the biosynthesis of ERα protein.

Taxol has many biological effects which appear to be related to its ability to promote the assembly of microtubules and to stabilize microtubules against depolymerization (Schiff & Horwitz 1980). The effect on the microtubule network produces a block in cell division which makes taxol a potent therapeutic drug against several types of cancer (Long 1994, Crown & O’Leary 2000, Mukherjee et al. 2001). However, the effects of taxol on ERα described in the present report are difficult to explain based exclusively on its effect on microtubules and may be related to an effect on signal transduction pathways. The increase in ERα gene transcription is rapid and occurs within 1 h of treatment with taxol, suggesting that the changes in expression or activity of signal transduction pathways rather than changes in microtubules are...
Figure 4 Transcriptional regulation of ERα promoter by taxol treatment. MCF-7 cells were grown as described in Fig. 1. (A) Effect of taxol on activity of the ERα promoter. MCF-7 cells were transiently transfected with a construct containing the first 128 bp of promoter A of the ERα gene driving the expression of the CAT reporter gene. CAT activity was determined by thin-layer chromatography. NT, control non-transfected cells; control, MCF-7 cells transfected with the CAT reporter gene; wild-type p53 (wp53), MCF-7 cells cotransfected with the CAT reporter gene and the wild-type p53 gene; taxol, MCF-7 cells transfected with the CAT reporter gene and treated with taxol. Triplicates of each condition are shown. (B) Effects of taxol on complex formation with the ERα promoter. MCF-7 cells were treated with 0.35 μM taxol at different incubation times and nuclear extracts were obtained as described in Materials and Methods. Binding of the 32P-labeled EP-3 oligonucleotide to nuclear extracts was determined by a DNA electrophoretic mobility shift assay. Lane 1, binding to nuclear extracts from untreated cells; lane 2, binding to nuclear extracts from cells treated with taxol for 1 h; lane 3, binding to nuclear extracts from cells treated with taxol for 6 h. Lanes 4 and 5 show nuclear extracts from taxol-treated cells competed with two different p53 antibodies. Lane 6 shows competition with an excess of cold EP-3 Arrow, specific binding of EP-3 to nuclear extracts. (C) As (B) except; lane 1, binding to nuclear extracts from untreated cells; lane 2, binding to nuclear extracts from cells treated with taxol for 6 h; lanes 3 and 4, binding to nuclear extracts from cells treated with taxol for 6 h competed with an excess of cold EP-2 and EP-1 respectively. Lane 5 shows competition with an excess of cold EP-3.
responsible for the increase in ERα transcription. However, it is not possible at the present time to rule out the possibility that the interaction of taxol with microtubules results in a rapid activation of signal transduction pathways. The dual effect of taxol on catecholamine secretion in chromaffin cells appears to involve both signal transduction pathways and microtubules (Thuret-Carnahan et al. 1985, Gomez et al. 2000). During the first hour of treatment, taxol induces an increase in catecholamine release, but at longer times the drug blocks the release of catecholamines (Thuret-Carnahan et al. 1985). The authors suggest that taxol has two different mechanisms of action. Initially, taxol induces an increase in intracellular Ca²⁺ concentration that triggers the increase in secretion observed within the first hour of treatment, whereas the later inhibitory effect is mediated by the effect of the drug on microtubules involved in the secretory process (Thuret-Carnahan et al. 1985).

The transcriptional effect of taxol on the ERα promoter may be due, in part, to the induction of p53. Similar to other studies (Tishler et al. 1995), taxol caused the rapid induction of p53, as we have demonstrated with the p53-EGFP chimera experiments. When transfected into MCF-7 cells, p53 increased transcription from the ERα promoter. In addition, p53 appears to interact with the complex that binds with the region of the ERα promoter involved in mediating the effects of taxol. However, there is no p53 consensus sequence within
this region, suggesting that the interaction of p53 with the ERα promoter is indirect. In support of an indirect mechanism, we have unpublished data demonstrating that p53 mutants that do not bind the DNA are able to induce ERα expression, while p53 mutations that interfere with protein–protein interactions block ERα induction.

Although the mechanisms involved in the taxol-induced inhibition of ERα translation are unknown, it is interesting to speculate that the effects of taxol may be mediated by TNF. Taxol is known to induce TNF (Burkhart et al. 1994), which in turn may repress translation through the phosphorylation of eukaryotic initiation factor-2α by the double-stranded RNA-activated protein kinase. It is also possible that the effects of taxol on ERα translation are independent of the TNF pathway. Taxol also affects the translation of tubulin mRNA. In the case of tubulin, the effects of taxol are due to an effect on tubulin mRNA half-life (Gong & Brandhorst 1988). Alternatively, the effects of taxol on the synthesis of ERα protein may be mediated by p53. The p53 protein is known to bind to the 5′ untranslated region of cdk4 and to mediate the inhibitory effects of transforming growth factor-β on the expression of cdk4 (Miller et al. 2000). It is also worth noting that cytoplasmic p53 is associated with a subset of ribosomes (Stein 1999).

ERα regulation in breast cancer cells is a complex phenomenon occurring at the transcriptional and post-transcriptional levels. Many factors including estradiol, anti-estrogens, phorbol esters, growth factors, and ions such as cadmium regulate the expression of ERα (Saceda et al. 1988, 1991, 1996, Lee et al. 1989, Cho et al. 1991, Garcia-Morales et al. 1994, Martin et al. 1995, Stoica et al. 1997, 2000a,b). Some of these compounds regulate ERα gene transcription, such as estradiol (Saceda et al. 1988, Cho et al. 1991), cadmium (Garcia-Morales et al. 1994), and gp30 (Saceda et al. 1996), while others affect the concentration of ERα through post-transcriptional mechanisms, e.g. estradiol (Saceda et al. 1988) and phorbol esters (Saceda et al. 1991). These findings suggest that ERα regulation is affected by many different stimuli in breast cancer cells. In fact, ERα could be regulated at the translational level because several upstream open reading frames have been described for this gene (Kos et al. 2002). For this reason, it is not difficult to imagine an effect of taxol on ERα levels. However, it still remains unclear as to whether the effects of the drug on the expression of ERα is the basis of its powerful response in hormone-regulated cancers such as breast carcinoma (Fornier et al. 2000).

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