

Repression of cancer protective genes by 17 β -estradiol: Ligand-dependent interaction between human Nrf2 and estrogen receptor α

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Abstract

Repression of cancer-protective phase II enzymes may help explain why estrogen exposure leads to the development of cancer. In an earlier report we described the ability of 17 β -estradiol (E₂) to repress phase II enzyme activity in vivo. Phase II enzymes are coordinately regulated via the presence of the antioxidant response element (ARE) in their promoter. We wanted to determine if estrogen receptors (ER) repress ARE-dependent gene expression through a mechanism that requires interaction with Nrf2, the transcription factor that regulates ARE-mediated gene transcription. E₂-bound ER α , but not ER β , represses ARE-regulated gene expression in the presence of exogenously expressed Nrf2 as well as when the transactivation domain of Nrf2 was fused to a heterologous DNA-binding domain. Deletion of the activation function-2 (AF-2) and the ligand-binding domain of ER α result in a constitutive repression of Nrf2-mediated transcription. Finally, E₂-bound ER α co-immunoprecipitates with Nrf2. Repression of Nrf2-mediated transcription by E₂-bound ER α expands our knowledge of E₂-regulated genes and provides a potential drug-screening target for the development of selective estrogen receptor modulators with a lower risk of causing cancer.

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1. Introduction

In this report we investigate the molecular mechanisms by which 17 β -estradiol (E₂) has been shown to repress phase II enzyme expression in vitro and in vivo (Ansell et al., 2004). Most chemical carcinogens are metabolized by two distinct classes of enzymes, commonly referred to as phase I and phase II enzymes. Phase I enzymes, which are members of the cytochrome P450 superfamily, convert many xeno-

biotic chemicals into highly reactive electrophilic products (Guengerich and Shimada, 1991; Cholerton et al., 1992; Korzekwa and Jones, 1993). Because of their ability to induce DNA damage and mutations, the highly reactive electrophilic products formed by phase I metabolism are responsible for the carcinogenic activity of many known chemical carcinogens, including DMBA and benzo(a)pyrene (Miller and Surh, 1994).

Phase II enzymes catalyze conjugation or reduction reactions that convert the highly reactive, carcinogenic chemicals produced during phase I metabolism to less reactive products. Phase II gene expression is coordinately regulated through a *cis*-acting DNA element referred to as the antioxidant/electrophilic response element (ARE/EpRE) which is

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located in the promoter of target genes (Rushmore et al., 1991). Chemical carcinogenesis can be inhibited by the consumption of certain electrophilic compounds, commonly referred to as chemopreventative agents (Wattenberg, 1978). This process, referred to as the electrophilic counter response (Prester et al., 1993), has been attributed to the ability of the chemopreventative agents to induce the expression of phase II enzymes. Induction of phase II enzyme expression protects against the development of cancer by increasing the ability of cells to detoxify and eliminate chemical carcinogens (Benson et al., 1978, 1980; Pantuck et al., 1979; Wattenberg, 1985). The induction of ARE-regulated genes occurs through the activation of the transcription factor Nrf2 (Itoh et al., 1997).

Under normal conditions, Nrf2 is sequestered in the cytoplasm by Kelch-like ECH-associated protein 1 (Keap1) and is shuttled for degradation (Itoh et al., 1999, 2003). Upon exposure to either a chemopreventative agent or oxidative stress, Keap1-mediated degradation of Nrf2 is diminished (Zhang and Hannink, 2003; Egger et al., 2005). Decreased degradation of Nrf2 leads to increased nuclear accumulation of Nrf2 that is able to dimerize with small maf transcription factors, bind to the ARE and induce transcription.

Through the use of animal models, it has clearly been demonstrated that altering the activity of Nrf2 and/or Nrf2-regulated phase II enzymes directly influences the development of cancer (Kwak et al., 2004). For example, in Nrf2-deficient animals, there are increases in DNA adducts in lungs after exposure to diesel exhaust (Aoki et al., 2001) and increases in gastric tumors after exposure to benzo(a)pyrene compared to wild type animals (Ramos-Gomez et al., 2001). Furthermore, the ability of the chemopreventative agent oltipraz to protect against the development of chemically induced cancer is lost in Nrf2-deficient animals (Ramos-Gomez et al., 2001). Due to the direct ability of Nrf2 and Nrf2-regulated phase II enzymes to influence the development of cancer, an understanding of signaling pathways that modulate Nrf2 activity could have great effects in regards to the chemoprevention of cancer.

E₂ exposure is a risk factor for the induction of breast, ovarian and uterine cancers. There are several distinct, coexisting mechanisms to explain how prolonged E₂ exposure leads to the development of cancer (Nicholson et al., 1999; Cavalieri et al., 2000; Ansell et al., 2004). The ability of E₂ to act as mitogens is one possible mechanism to explain how estrogen exposure contributes to cancer development. A second mechanism proposed to explain how E₂ exposure leads to the development of cancer is the formation of DNA adducts and DNA mutations by chemically reactive E₂ metabolites (Liehr, 1990).

A third mechanism proposed to explain how E₂ exposure leads to the development of cancer is the repression of cancer protective phase II enzymes by E₂. Following up on the work of Montano et al., who first demonstrated antagonist bound estrogen receptors (ER)-signaling through an ARE-mediated pathway (Montano and Katzenellenbogen, 1997; Montano et al., 1998), we showed that E₂ treatment repressed ARE-

dependent, phase II enzyme activities in cell culture and in the uterus of mice, in effect mimicking the situation observed in Nrf2-deficient mice (Ansell et al., 2004). Decreased phase II enzyme activity would slow the metabolism of nucleophilic compounds generated by endogenous sources such as 4-hydroxyestradiol as well as exogenous sources such as environmental chemicals and thereby impair the ability of cells to detoxify and eliminate chemical carcinogens before they cause cancer-causing DNA lesions.

In this report, we asked if E₂-bound estrogen receptors α and ER β repress ARE-dependent gene expression through an Nrf2-mediated mechanism. Our results indicate that E₂-bound ER α , but not ER β , is able to repress Nrf2-mediated transcription. The ability of E₂-bound ER α to regulate gene expression through an interaction with an Nrf2-mediated ARE pathway increases our knowledge of E₂-regulated target genes and provides a potential drug-screening target for the development of selective estrogen receptor modulators (SERMs) with a lower risk for causing cancer.

2. Materials and methods

2.1. Chemicals

All chemicals and hormones were obtained from Sigma Chemical Co. (St. Louis, MO) unless otherwise stated.

2.2. Plasmids

The vectors containing Gal4-luciferase, the 4X ARE reporter, hemagglutinin (HA) tagged Nrf2 and the Nrf2 transactivation domain-Gal4 DNA-binding domain fusion vector have been described previously (Zhang and Hannink, 2003). For the Nrf2 transactivation domain-Gal4 fusion protein, amino acids 98–454 of human Nrf2 were fused to the Gal4-DNA-binding domain in a pcDNA3-derived vector using standard recombinant DNA techniques. The dominant negative Nrf2 vector has been described previously (Alam et al., 1999).

The full length ER α and ER β expression vectors have been described previously (Ansell et al., 2004). The ER deletion constructs HE 11, HE 14, HE 15 and HE 19, kindly provided by Dr. Pierre Chambon, have been described previously and have been shown to be expressed in equivalent concentrations when expressed from the same promoter (Kumar et al., 1986, 1987). To ensure equivalent expression with the full length ER α , the ER α deletion fragments were subcloned into pCDNA3.1 (Invitrogen, Carlsbad, CA) using standard techniques and verified by DNA sequence analysis (University of Missouri DNA Core). The ER α deletion constructs contain a G400V mutation present from the original cloning of ER α . By comparing a full length ER α construct containing V at position 400 to a full length construct containing G at amino acid 400, it was determined that the amino acid substitution did not affect the ability to regulate

ARE-mediated and Nrf2-mediated transcription (data not shown).

2.3. Cell culture and transfection experiments

Ishikawa cells were maintained in complete medium consisting of phenol red-free Eagle's Minimal Essential Medium supplemented with insulin (6 ng/ml), HEPES (10 mM), and 10% charcoal stripped fetal bovine serum (Life Technologies Inc., Gaithersburg, MD). COS1 cells were maintained in Dulbecco's Modified Eagle's Medium (Mediatech Inc., Herndon, VA) containing 10% fetal bovine serum (Life Technologies Inc.). For transient transfection experiments, cells were plated in 24 well plates and transiently transfected using plus and lipofectamine reagents (Invitrogen). Transfection experiments were normalized to the co-transfected PHRG-TK control renilla vector. Luciferase assays were done using the dual luciferase assay kit (Promega, Madison, WI).

All experiments were performed at least three times with duplicate samples per experiment. Results from three experiments were averaged together and shown with error bars as standard error of the mean. Statistical significance was determined using a *T*-test.

2.4. Co-immunoprecipitation

COS1 cells were transfected with 2 μ g of either ER α or HA-Nrf2 and allowed to recover overnight. The following day, cells were treated with or without 10 nM E₂ and incubated overnight. The following day, cells were lysed with complete ELB buffer which consisted of 50 mM HEPES, 250 mM NaCl, 5 mM EDTA, 0.1% triton X-100, protease inhibitor cocktail (Sigma), phosphatase inhibitor (Sigma) and 2.5 mM DTT. The lysates were microcentrifuged at top speed for 20 min, soluble supernatants were then combined and immunoprecipitated using anti-HA antibody (Santa Cruz Biotechnologies, Santa Cruz, CA) and protein A beads (Sigma). The presence of ER α was detected by Western blot analysis.

3. Results

3.1. Regulation of antioxidant response element-mediated gene expression by 17 β -estradiol in COS1 cells

Previously, we have shown that E₂ treatment would repress the activities of ARE-regulated gene expression in COS1 cells as well in mouse uterus (Ansell et al., 2004). Due to the central importance of Nrf2 in regulating ARE-dependent gene expression, we wanted to determine if ER α and ER β repress ARE-dependent transcription through an Nrf2-mediated mechanism in COS1 cells as well as in the uterine-derived Ishikawa cells.

COS1 cells were transiently transfected with a 4X mouse GST A1 (Ya) luciferase reporter in the absence or pres-

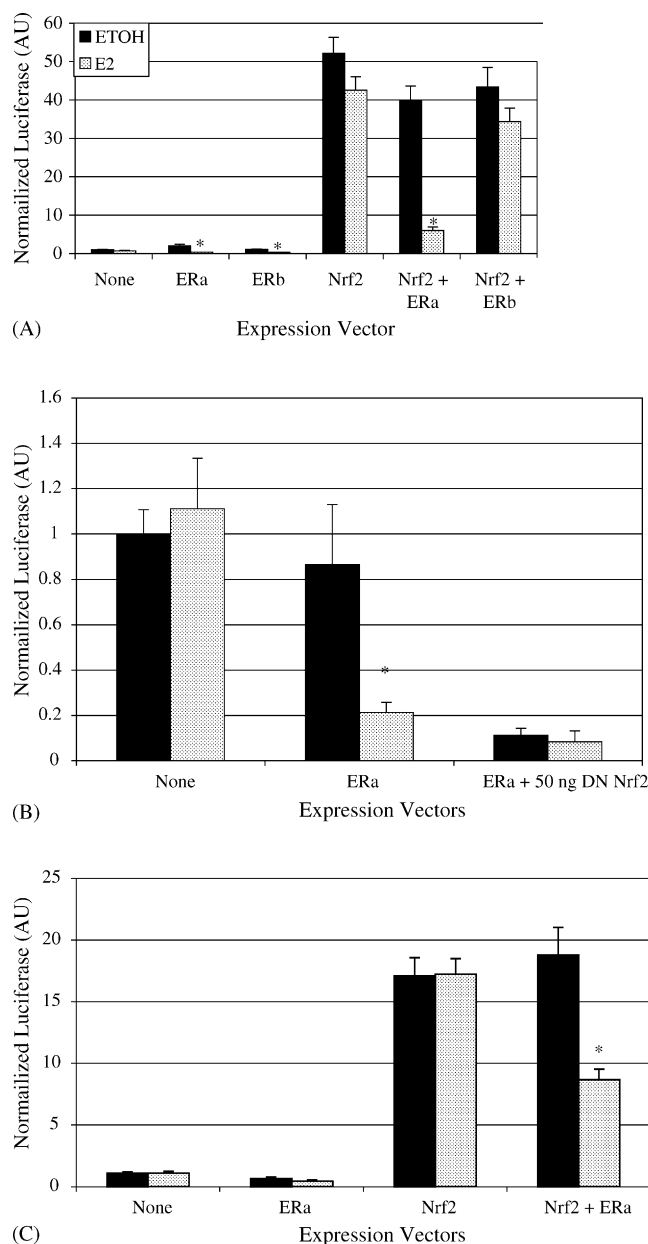


Fig. 1. E₂-mediated regulation of a 4X ARE reporter gene in COS1 and Ishikawa cells. Cells were transfected with the expression vectors listed on the X-axis and allowed to recover overnight. For (A) and (C), 2 ng of HA-Nrf2 and 10 ng ER were transfected into each well. For (B), 50 ng ER α and 50 ng dominant negative Nrf2 were transfected into each well. After recovery, cells were treated for 24 h with or without 10 nM E₂, lysed and then analyzed for luciferase activity. Experiments were performed three times with duplicate samples per experiment, results from all three experiments were averaged and statistical significance was calculated using a *T*-test. Asterisks indicate *p* < 0.05. (A) and (B) show the results of experiments performed in COS1 cells; (C) shows the results of experiments performed in an ER-negative variant Ishikawa cell line.

ence of co-transfected ER α or ER β . After transfection, cells were treated with ethanol vehicle or 10 nM E₂, and the effect of E₂ treatment on the regulation of the reporter construct was determined (Fig. 1A). E₂ treatment was able to repress ARE-dependent gene transcription in the presence

of co-transfected ER α and ER β in COS1 cells as previously reported (Ansell et al., 2004). An Nrf2 expression vector was co-transfected into COS1 cells along with either ER α or ER β and it was determined that upon E₂ treatment, ER α , but not ER β , could repress ARE-mediated gene transcription in the presence of exogenously expressed Nrf2 (Fig. 1A).

To determine if ER α can repress ARE activity in the absence of Nrf2, a dominant negative Nrf2 expression vector was used to block the activity of endogenous Nrf2. As expected, transfection of COS1 cells with the dominant negative Nrf2 expression vector resulted in the repression of basal ARE-mediated transcription (Fig. 1B). E₂ treatment was unable to repress ARE-mediated gene transcription in the presence of dominant negative Nrf2 (Fig. 1B) suggesting that Nrf2 is required for E₂-mediated repression of ARE transcription.

3.2. Regulation of antioxidant response element-mediated gene expression by 17 β -estradiol in Ishikawa cells

Next, we wanted to determine if ER α -mediated repression of Nrf2 was cell-type specific or could occur in additional cell types. In our previous work, a variety of mouse tissues were screened to determine which tissues E₂ could repress ARE-regulated phase II enzyme activity. The uterus was found to be the most responsive to E₂-mediated repression of ARE-regulated gene expression (Ansell et al., 2004). Therefore, the uterine-derived Ishikawa cell line was used to study the molecular mechanism(s) of E₂-mediated repression of ARE-dependent gene expression.

Ishikawa cells were transiently transfected with a 4X mouse GST A1 (Ya) luciferase reporter, and the effect of E₂ treatment on the regulation of the reporter construct was determined (Fig. 1C). Because E₂ treatment was unable to regulate expression of the 4X ARE reporter gene, we suspected that our Ishikawa cell line was ER-negative (Nishida, 2002) so an expression vector for ER α was transfected into the Ishikawa cells. E₂ treatment was unable to alter the expression of the ARE reporter gene even in the presence of co-transfected ER α when compared to the ethanol (ETOH) treated control (Fig. 1C).

Ishikawa cells contain very low levels of Nrf2 due to proteasomal-mediated degradation of Nrf2 by Keap1 (Shih-Ching Lo and Mark Hannink, unpublished results). We hypothesized that the low abundance of Nrf2 in Ishikawa cells may account for the inability of E₂ treatment to repress ARE-dependent gene transcription. To test this hypothesis, Nrf2 was co-transfected into Ishikawa cells. As expected, Nrf2 expression resulted in a potent upregulation of ARE-dependent gene expression (Fig. 1C). E₂ treatment did not affect Nrf2-mediated regulation of ARE-dependent gene expression in the absence of co-transfected ER α , but E₂ treatment repressed ARE-dependent gene expression when both Nrf2 and ER α were co-expressed (Fig. 1C).

3.3. Regulation of a Gal4–Nrf2 fusion protein by ER α and ER β in COS1 cells

Nrf2 contains several functional domains. The N-terminus of Nrf2 contains a hydrophilic domain termed Neh2 that interacts with the protein Kelch-like ECH-associated protein 1 (Itoh et al., 1999). The central region of Nrf2 contains a potent transcriptional activation domain. The carboxy-terminus of Nrf2 contains a bZip domain responsible for nuclear import, DNA binding and dimerization.

To determine if the transactivation domain of Nrf2 is the target of E₂-mediated repression, the transcriptional activation domain of Nrf2 was fused to a heterologous DNA-binding domain. As a negative control, the Gal4-DNA-binding domain was expressed in COS1 cells along with the Gal4-luciferase reporter gene. Compared to untreated cells, E₂ treatment was unable to repress expression of the Gal4-driven reporter construct in the absence or presence of co-expressed ER α or ER β (Fig. 2A). The low-level background stimulation of the Gal4-luciferase reporter seen when ER α was added with the Gal4DBD was not statistically significant.

Expression of Gal4–Nrf2 in COS1 cells results in a potent induction of Gal4–Nrf2-mediated transcription when compared to cells not transfected with Gal4–Nrf2 (compare Y-axis scale in Fig. 2A and B). In the absence of co-transfected ER α , E₂ treatment was unable to alter Gal4–Nrf2-mediated transcription. Upon co-expression of Gal4–Nrf2 with ER α , E₂ treatment repressed Gal4–Nrf2-mediated gene expression when compared to untreated cells. ER β was unable to regulate Gal4–Nrf2-mediated gene expression in the presence of E₂. These results indicate that ER α , but not ER β , can repress function of the transcriptional activation domain of Nrf2.

3.4. Regulation of a Gal4–Nrf2 fusion protein by ER α in Ishikawa cells

To determine if the transactivation domain of Nrf2 is the target of E₂-mediated repression in Ishikawa cells, the Gal4-DNA-binding domain Nrf2 fusion construct was employed. As a negative control, the Gal4-DNA-binding domain was expressed in Ishikawa cells along with the Gal4-luciferase reporter gene. E₂ treatment was unable to alter the expression of the Gal4-driven reporter construct in the absence or presence of co-expressed ER α (Fig. 2C).

Expression of Gal4–Nrf2 in Ishikawa cells results in a potent induction of Gal4–Nrf2-mediated transcription (compare Y-axis scale in Fig. 2C and D). In the absence of co-transfected ER α , E₂ treatment was unable to alter Gal4–Nrf2-mediated transcription. Upon co-expression of Gal4–Nrf2 with ER α , E₂ treatment repressed Gal4–Nrf2-mediated gene expression when compared to untreated cells. These results indicate that the transcriptional activation domain of Nrf2 is the target of E₂-mediated repression of Nrf2-dependent transcription in Ishikawa cells.

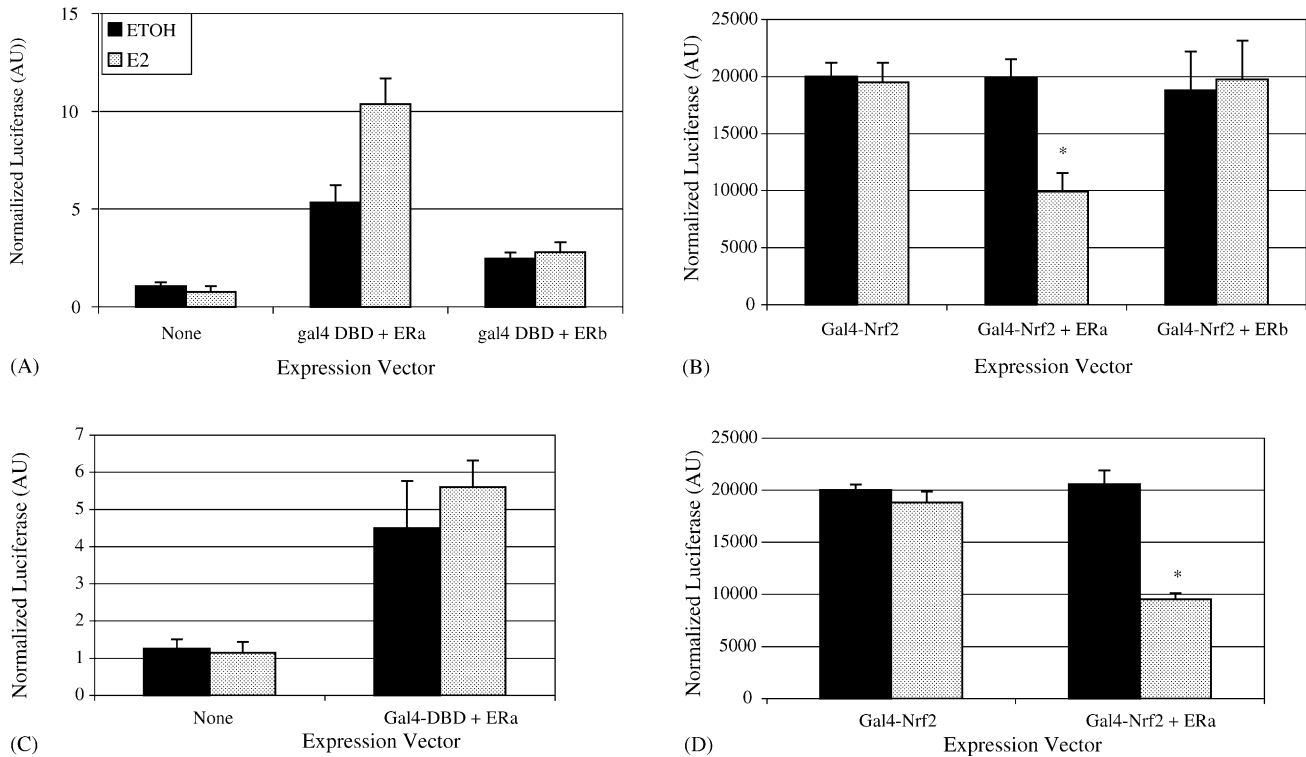


Fig. 2. Regulation of the transcriptional activation domain of Nrf2 in COS1 and Ishikawa cells. Cells were transiently transfected with a Gal4-luciferase reporter gene, a renilla control vector and 1 ng of the expression vectors listed on the X-axis. Cells were treated for 24 h with 10 nM E₂, lysed and analyzed for luciferase activity. (A) Control data showing that E₂ treatment cannot repress expression of the Gal4-luciferase reporter vector in the absence or presence of ERs and in the presence of the Gal4-Nrf2 vector which contains amino acids 98–454 of human Nrf2 fused to the Gal4-DNA-binding domain in COS1 cells; (B) experimental data showing that E₂ treatment can repress the activity of the transcriptional activation domain of Nrf2 fused to the Gal4-DNA-binding domain in the presence of ER α but not ER β in COS1 cells; (C) control data showing that E₂ treatment cannot repress expression of the Gal4-luciferase reporter vector in the absence or presence of ER α and in the presence of the Gal4-DNA-binding domain expression vector in Ishikawa cells; (D) experimental data showing that E₂ treatment can repress the transcriptional activation domain of Nrf2 fused to the Gal4-DNA-binding domain in a ligand-dependent fashion in the presence of ER α in Ishikawa cells. Experiments were performed three times with duplicate samples per experiment, the results from all three experiments were averaged together and statistical significance was calculated using a *T*-test. Asterisks indicate $p < 0.05$.

3.5. Regulation of antioxidant response element-mediated gene expression by ER α deletion constructs in Ishikawa cells

To determine the domain(s) of ER α required for repression of Nrf2-dependent gene transcription, a series of deletion constructs lacking specific domains of ER α were employed (Fig. 3). ER α is a modular protein consisting of multiple domains termed A through F. The N-termini of ER α , termed the A/B domain, contains the ligand-independent activation function-1 (AF-1). The central region of ER α , termed the C domain, contains the DNA-binding domain. The D domain, which is classically referred to as the hinge region, is located between the DNA-binding domain and the ligand-binding domain. The E domain, which is located C-terminal to the D domain, contains the ligand-binding domain and activation function-2 (AF-2). The F domain, which is located adjacent to the E domain, influences transcriptional activation in the presence of certain ligands (Montano et al., 1995).

Expression vectors containing wild-type ER α or various ER α deletion constructs were co-transfected into Ishikawa cells along with full-length Nrf2 to determine which domains

of ER α were required to repress Nrf2-dependent transcription in the absence or presence of 10 nM E₂. In the absence of E₂ treatment, the construct lacking AF-2 and the ligand-binding domain (ER 15) constitutively repressed Nrf2-dependent transcription whereas all other constructs used had no effect on Nrf2-mediated transcription (Fig. 3).

All ER α deletion constructs except the construct lacking the A/B and C domains (ER 14) repressed Nrf2-mediated transcription in the presence of 10 nM E₂. ER 11, which lacks the C domain and ER 19, which lacks the A/B domains, both repress Nrf2-mediated transcription in a ligand-dependent fashion (Fig. 3).

3.6. Ligand-dependent interaction between ER α and Nrf2

To determine if ER α and Nrf2 interact in a ligand-dependent manner, a co-immunoprecipitation experiment was performed. COS1 cells were transiently transfected with either ER α or Nrf2, lysates prepared and then mixed together in the absence or presence of E₂. Total lysate blots were performed to determine that each immunoprecipita-

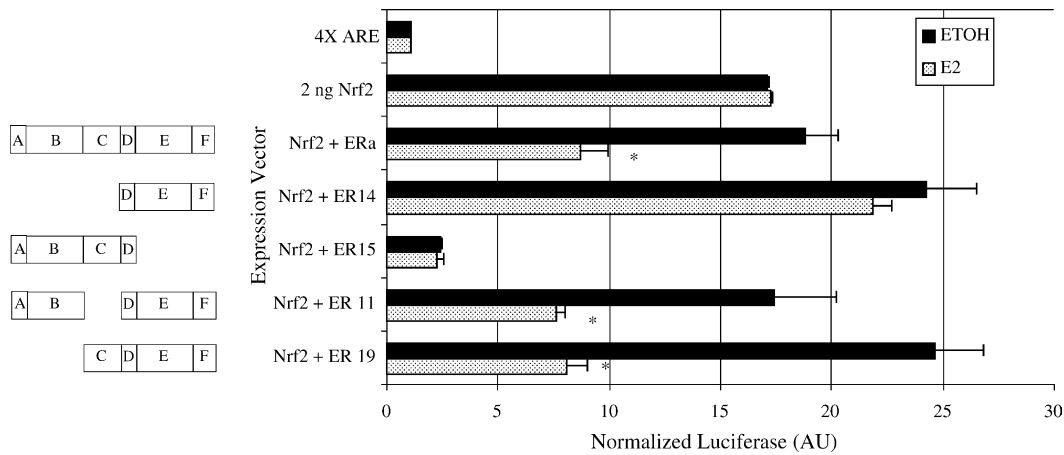


Fig. 3. Regulation of Nrf2 transcriptional activity by various ER α deletion constructs. Ishikawa cells were transiently transfected with a 4X ARE reporter gene, a renilla control vector and the expression vectors listed on the Y-axis. 2 ng of HA-Nrf2, 10 ng wild type ER α or 50 ng of each ER α deletion construct was transfected into each well. Cells were treated for 24 h with 10 nM E₂, lysed and analyzed for luciferase activity. The experiment was performed four times with duplicate samples per experiment. Results from a representative experiment are shown. Asterisks indicate $p < 0.05$.

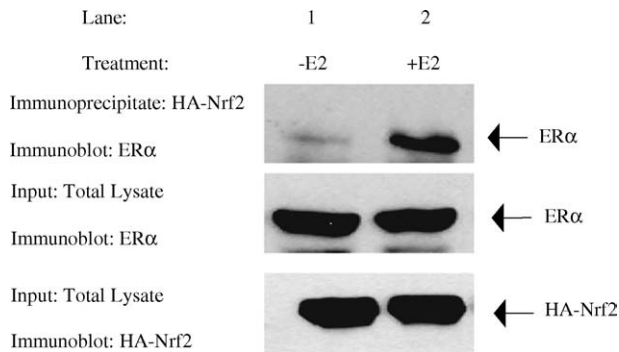


Fig. 4. Ligand-mediated interaction between ER α and Nrf2. COS1 cells were transfected with either ER α or HA-Nrf2. Cells were lysed and the lysates were mixed together in the absence (lane 1) or presence (lane 2) of E₂. HA-Nrf2 interacting proteins were immunoprecipitated using an anti-HA antibody and the presence of ER α was detected using Western blot analysis. Prior to immunoprecipitation, total lysates were blotted to confirm equivalent expression levels of ER α or HA-Nrf2 in the absence (lane 1) or presence of E₂ (lane 2).

tion sample had equivalent amounts of ER α and HA-Nrf2 (Fig. 4).

The mixed lysates were immunoprecipitated using an anti-HA antibody, and Western blot analysis was performed to detect the presence of ER α . E₂ treatment increased the levels of ER α that was detected in the HA-immunoprecipitated samples (Fig. 4). In the absence of co-transfected HA-Nrf2, ER α was undetected by Western blot analysis after precipitation with the HA antibody (data not shown). This result indicates that the immunoprecipitation result was not due to a nonspecific interaction between ER α and the HA antibody.

4. Discussion

Nrf2-regulated phase II enzymes protect against the development of cancer by catalyzing reactions that convert highly

reactive, carcinogenic chemicals to less reactive products. In this report, we have characterized a molecular mechanism by which 17 β -estradiol represses ARE-dependent gene expression. Repression of phase II enzymes by estrogen through an ARE-dependent pathway could help explain why estrogen exposure is a risk factor for the development of cancer.

When uninduced, Nrf2 is targeted for degradation by Keap1 (Itoh et al., 2003). Upon exposure to a chemical carcinogen or a chemoprotective chemical, Nrf2 escapes degradation, accumulates in the nucleus, dimerizes with small maf transcription factors and activates ARE-dependent gene expression (Zhang and Hannink, 2003; Eggler et al., 2005). Several possible mechanisms exist in which E₂-liganded ER α could repress Nrf2-mediated transcription. The first potential mechanism by which E₂-liganded ER α could repress Nrf2-mediated transcription is through increasing Keap1-mediated degradation of Nrf2. The second mechanism by which E₂-liganded ER α could repress Nrf2-mediated transcription is through direct competition for overlapping DNA binding sites. A third possible mechanism by which E₂-liganded ER α could repress Nrf2-mediated transcription is through a ligand-dependent, direct interaction of ER α with Nrf2 that results in the repression of ARE signaling.

The Gal4-Nrf2 fusion protein used in this report lacks the Keap1-interacting domain and the DNA-binding/maf dimerization domains of Nrf2. The ability of ER α to repress the function of an Nrf2 protein that lacks the DNA-binding and Keap1-interacting domains implies that ER α does not repress Nrf2-dependent transcription by increasing Keap1-mediated degradation of Nrf2 or by direct competition for overlapping DNA-binding sites. The data obtained using the Gal4-Nrf2 fusion protein do support a mechanism in which E₂-bound ER α represses ARE-dependent transcription by repressing the function of the transcriptional activation domain of Nrf2. Furthermore, the immunoprecipitation data determined that there is a ligand-dependent physical interaction between ER α and Nrf2.

Through the use of ER α deletion constructs, we attempted to determine which domains of ER α are required to repress Nrf2-mediated transcription. An ER α deletion construct lacking the A, B and C domains (ER 14) was unable to repress Nrf2 signaling in the absence or presence of E₂, likely indicating that the sequences required to repress Nrf2-mediated transcription are not located in the D, E and F domains of ER α . The ability of ER 15 to act as a constitutive repressor of Nrf2-mediated transcription likely indicates that sequences located in the A/B or C domains of ER α are responsible for repressing Nrf2-mediated transcription.

To determine if the domain capable of repressing Nrf2-mediated transcription is located in the A/B domain, which contains AF-1, or in the C domain, which contains the DNA binding domain, deletion constructs lacking each domain was used. In the absence of the C domain (ER 11) or in the absence of the AF-1 domain (ER 19), each construct repressed Nrf2-mediated signaling in the presence of E₂. The ability of both ER 11 and ER 19 to repress Nrf2-mediated transcription in a ligand-dependent manner implies that either the A/B or the C domains are capable of repressing Nrf2 signaling. The ability of ER α to interact with a given protein through several domains has been reported for other estrogen-mediated signaling pathways (Likhite et al., 2004). Smaller deletions of ER α will need to be performed to more accurately determine which domains of ER α are required to repress Nrf2-mediated transcription.

Estrogen regulation of Nrf2-mediated transcription expands our knowledge of estrogen-regulated target genes to those that are regulated by the ARE or Nrf2. Nrf2 regulates the expression of many classes of genes including metabolic enzymes, NADPH-regenerating enzymes, hydrolysis, reduction, oxidation, glucuronidation, glutathione transferases and glutathione synthesis pathway (Thimmulappa et al., 2002). Additionally, it has been reported that aromatase, the enzyme responsible for synthesizing E₂, contains an ARE in its promoter (Wasserman and Fahl, 1997).

In COS1 cells, in the absence of exogenous Nrf2, E₂ is able to repress ARE-mediated gene expression through ER α as well as ER β . The inability of ER β to significantly repress Nrf2-mediated transcription in the presence of exogenous Nrf2 and when Nrf2 was tethered to a heterologous promoter could have several different explanations. The most likely explanation is that ER β 's binding affinity for Nrf2 is much lower than ER α so that more ER β needs to be added to get an equivalent effect.

Anti-estrogen-bound ER β has been shown to be in a complex with Nrf2 and to upregulate ARE-mediated transcription (Montano et al., 2004), but the different conformations adopted by ERs when bound to differing ligands may affect the ability of ER β to bind Nrf2. Thus, a second potential explanation as to why E₂-bound ER β does not regulate exogenous Nrf2-mediated transcription is that E₂-bound ER β may use different cell-specific factors to repress ARE-mediated transcription than anti-estrogen-bound ER β does to activate ARE-mediated transcription.

In conclusion, we present evidence for ER α ' ability to regulate ARE-mediated gene expression through an Nrf2-mediated mechanism. This increases our understanding of ER-regulated target genes and adds an additional mechanism by which estrogens can help regulate diverse physiological functions involved in cancer, reproductive physiology and metabolism.

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