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Activation of estrogen receptor α by raloxifene through an activating protein-1-dependent tethering mechanism in human cervical epithelial cancer cells: A role for c-Jun N-terminal kinase

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ABSTRACT

Nuclear estrogen receptor α (ERα) regulates target gene expression in response to ligands through two distinct mechanisms: direct binding to DNA and indirect tethering through other DNA-bound transcription factors, such as AP-1. In the studies described herein, we examined the molecular mechanisms underlying the activation of ERα in the AP-1 tethering pathway by the selective estrogen receptor modulator (SERM) raloxifene (Ral). Our results with the *MMP1* and *PRUNE* genes indicate that the c-Fos component of the AP-1 tethering factor and the c-Jun N-terminal kinase 1 (JNK1) are constitutively bound at the promoter regions prior to Ral exposure. Ral then promotes the binding of ERα at the promoter in a c-Fos-dependent manner. Interestingly, we found that JNK1 enzymatic activity is required for Ral-dependent gene activation through ERα. Our results suggest that one role for Ral-dependent recruitment of ERα to the AP-1 binding site is to stimulate JNK1 enzymatic activity. Alternatively, Ral-occupied ERα might recruit protein substrates to promoter-bound JNK1 without any change in JNK1 activity. Collectively, our studies have revealed a new role for JNK1 in determining gene regulatory outcomes by ERα.

1. Introduction

Estrogens control physiological and pathophysiological processes in a wide variety of tissues, exerting their effects through two distinct estrogen receptor proteins, ERα and ERβ (Heldring et al., 2007; Warner et al., 1999). ERs act as ligand-regulated, DNA-binding transcription factors that regulate distinct subsets of genes across the genome (Cheung and Kraus, 2010; Heldring et al., 2007; Kininis and Kraus, 2008; Warner et al., 1999). ERs can bind directly to genomic DNA as dimers through specific sequences called estrogen response elements (EREs). ERs can also be recruited to genomic DNA by indirect tethering through other DNA-bound transcription factors, including members of the activating protein-1 (AP-1) family of transcription factors (e.g., heterodimers of c-Jun and c-Fos) (Heldring et al., 2007, 2011; Kushner et al., 2000). In either case, chromatin associated ERs nucleate the recruitment of a variety of coregulator proteins (e.g., coactivators and corepressors) to activate or repress gene transcription (Acevedo and Kraus, 2004; Glass and Rosenfeld, 2000). By now, hundreds of potential coregulators with diverse functions – from histone modification and chromatin remodeling to RNA polymerase II recruitment and mRNA splicing – have been identified (Lonard and O'Malley, 2006). The specific requirements for and functions of these coregulators, and their modulation by different ER ligands, have not been fully defined.

A wide variety of both natural and synthetic ER ligands have been identified and characterized. Selective estrogen receptor modulators (SERMs), such as tamoxifen (Tam) and raloxifene (Ral), are a class of ER ligands that exhibit context-specific agonist and antagonist activities (Kuiper et al., 1999; McDonnell et al., 2001). For example, Tam and Ral act predominantly as ER antagonists in the mammary glands, but exhibit pronounced agonistic effects in other tissues, including bone (Tam and Ral) and uterus (Tam only) (Kuiper et al., 1999; McDonnell et al., 2001; Shang and Brown, 2002). The ER/AP-1 tethering pathway is activated in response to both estrogens and SERMs in a cell-specific and ER isoform-specific manner (Kushner et al., 2000; Webb et al., 1995). In some cellular contexts, 17β-estradiol (E2), the predominant naturally occurring estrogen, activates ERα, but not ERβ, in the tethering pathway, while Tam and Ral may activate both ER isoforms in the tethering pathway (Paech et al., 1997). The different ligand

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responses observed with different cellular contexts, different modes of ER recruitment to genomic DNA (i.e., ERE versus tethering), and different ER isoforms (i.e., ERα versus ERβ) are likely due to differential use of coregulator proteins (Cheung et al., 2005; Kushner et al., 2000; Webb et al., 2003).

c-Jun N-terminal kinase 1 (JNK1) is a mitogen-activated protein kinase that interacts with and phosphorylates c-Jun in response to cellular signaling pathways (Barr and Bogoyevitch, 2001; Dunn et al., 2002). Although the traditional view has been that MAPK-mediated phosphorylation events (e.g., phosphorylation of transcription factors) do not occur at the downstream target genes that they ultimately regulate, the terminal kinases of various signaling pathways are found in the nucleus under activating conditions (Edmunds and Mahadevan, 2004; Turjanski et al., 2007). In addition, gene-specific and genomic analyses in cells from yeast (Pascual-Ahuir et al., 2006; Pokholok et al., 2006), *Drosophila* (Suganuma et al., 2010), and mammals (Bruna et al., 2003; Bungard et al., 2010; Dawson et al., 2009; Edmunds and Mahadevan, 2004; Hu et al., 2009; Madak-Erdogan et al., 2011; Vicent et al., 2006) have shown that some signaling kinases bind to the promoters of genes whose expression they regulate. In the studies described herein, we used cell-based reporter assays, gene-specific mRNA analyses, and chromatin immunoprecipitation (ChIP) assays to explore SERM-dependent activation of ERα in the AP-1 tethering pathway and characterize the role of JNK1 in this pathway. Our results indicate that JNK1 enzymatic activity plays a key role in supporting Ral agonistic actions in the tethering pathway for some genes.

2. Materials and methods

2.1. Antibodies

The JNK1/3 antibody was from Santa Cruz Biotechnology (sc-474). The custom rabbit polyclonal antisera against c-Fos and ERα were produced by Pocono Rabbit Farms and Laboratory.

2.2. Plasmid DNA constructs

The following luciferase reporter plasmids were used in these studies: (1) MMP1-Luc, which was constructed by cloning the −73 to +63 fragment of the human collagenase/matrix metalloproteinase-1 (MMP-1) gene (Angel et al., 1987) upstream of the luciferase reporter gene in the pXP1 reporter plasmid (Nordeen, 1988) (provided by Dr. Steve Nordeen, University of Colorado Health Sciences Center), (2) pGL3-2ERE-pS2-Luc (referred to herein as 2ERE-TFF1-Luc), which contains two estrogen response elements (EREs) and a fragment of the pS2/trefoil factor 1 promoter upstream of a luciferase reporter gene (Kim et al., 2006), (3) PRUNE-Luc, which contains the −461 to +55 bp fragment of the PRUNE promoter upstream of a luciferase reporter gene (kindly provided by Dr. Miltos Kininis from the Kraus laboratory), and (4) UGT2B15-Luc, which contains the −449 to +114 bp fragment of the *UGT2B15* promoter upstream of the luciferase reporter (Kininis et al., 2007). The *MMP1*, *PRUNE*, and *UGT2B15* promoter fragments lack identifiable EREs, but contain an AP-1 binding site, as illustrated in Fig. 1A.

The following expression vectors were used: (1) pRSV, an "empty" expression vector containing the Rous sarcoma virus promoter, (2) RSV-hERα, which expresses human estrogen receptor alpha (provided by Dr. Benita Katzenellenbogen, University of Illinois, Urbana-Champaign) (Reese and Katzenellenbogen, 1991), (3) pCMV, an "empty" expression vector containing the cytomegalovirus promoter, (4) pCMV-A-Fos, which expresses a dominant-negative version of c-Fos (provided by Dr. Charles Vinson, NCI, and Nina Heldring from the Kraus laboratory) (Heldring et al., 2011; Olive et al., 1997), and (5) pCMVβ, a constitutive β-galactosidase expression vector used for transfection normalization (Clontech).

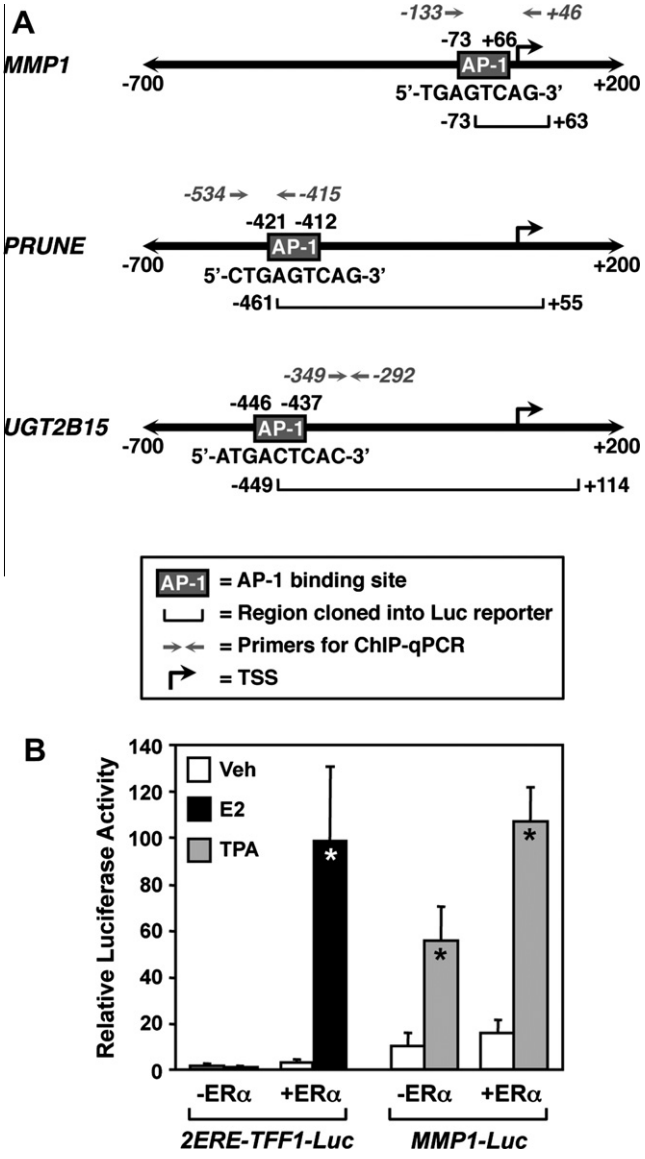

Fig. 1. Schematic diagrams of the promoters used for the luciferase, gene expression and ChIP assays, and verification of the experimental system. (A) The structures of the *MMP1*, *PRUNE*, and *UGT2B15* promoters from −700 to +200 bp relative to the transcription start site (TSS) are shown. The locations of the known AP-1 binding sites (boxes labeled "AP-1"), promoter DNA fragments used in the luciferase reporters (horizontal brackets), and locations of the primers used for ChIP-qPCR are indicated. The bent arrow denotes the TSS for each gene. (B) E2 and TPA activate their cognate reporter genes in HeLa cells. An E2-responsive luciferase reporter vector (2ERE-TFF1-Luc) or a TPA-responsive reporter vector (MMP1-Luc) was transfected into HeLa cells with or without an expression vector for ERα. The cells were then treated with E2 (100 nM) or TPA (25 ng/mL TPA), as indicated, before determination of luciferase activity. Each bar represents the mean ± SEM for $n \geqslant 4$ separate experiments. Bars marked with an asterisk are significantly different from the corresponding controls (Student's *t*-test, $p < 0.05$).

2.3. Cell growth and maintenance

HeLa cells were purchased from ATCC. HeLa–ERα cells were kindly provided by Dr. David Shapiro (University of Illinois, Urbana-Champaign) (Zhang et al., 1999). Both cell types were