Multiple Transcription Factor Elements Collaborate with Estrogen Receptor α to Activate an Inducible Estrogen Response Element in the NKG2E Gene

Nitzan Levy, Xiaoyue Zhao, Hui Tang, Robert B. Jaffe, Terence P. Speed, and Dale C. Leitman

Departments of Obstetrics, Gynecology, and Reproductive Sciences, Center for Reproductive Sciences (N.L., R.B.J., D.C.L), and Cellular and Molecular Pharmacology (N.L., D.C.L), University of California, San Francisco, California 94143; Department of Statistics (X.Z, H.T., T.P.S.), University of California, Berkeley, California 94720; and Division of Genetics and Bioinformatics (T.P.S.), The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria 3052, Australia

Estrogen receptors (ERs) regulate transcription by interacting with regulatory elements in target genes. However, known ER regulatory elements cannot explain the expression profiles of genes activated by estradiol (E2) and selective estrogen receptor modulators (SERMs). We previously showed that the killer cell lectin-like receptor (NKG2E) gene is regulated by E2, tamoxifen, and raloxifene. Here we used the NKG2E gene as a model to investigate the mechanism whereby target genes are regulated by E2 and SERMs with ERs. The ER regulatory element in the NKG2E promoter was mapped to the –1825 and –1686 region. Full activation of the NKG2E promoter required the collaboration between a transcription factor cluster containing c-jun, heat-shock factor 2, and CCAAT/enhancer-binding protein β and a unique variant estrogen response element (ERE) that has only a two nucleotide spacer between half sites. The cluster elements and the variant ERE were inactive on their own, but the regulation by E2 and SERMs was restored when the c-jun, heat-shock factor 2, and CCAAT/enhancer-binding protein β cluster was placed upstream of the variant ERE. The activation of the NKG2E gene by E2 and selective ER modulators was associated with the recruitment of the p160 coactivators glucocorticoid receptor-interacting protein 1 and amplified in breast cancer 1 but not steroid receptor co-activator 1. These studies identified one of the most complex ER regulatory units thus far reported and demonstrate that a cluster of flanking transcription factors collaborate with ER to induce a functional ERE in the NKG2E promoter. (Endocrinology 148: 3449–3458, 2007)

Estrogens and selective estrogen receptor modulators (SERMs) induce physiological and clinical effects by interacting with estrogen receptors (ERs), which are members of a large family of proteins known as nuclear receptors (1, 2). Two types of ERs have been identified, ERα and ERβ (3, 4). These receptors have different tissue distributions and biological roles. ERs act through two signaling pathways: a genomic pathway, in which ERs in the nucleus regulate gene transcription (5), and a nongenomic pathway, in which ERs located in the plasma membrane regulate signal transduction (6). Binding of estradiol (E2) to nuclear ER induces a conformational change of the receptor that allows it to directly attach to an estrogen response element (ERE) (7). Once the liganded ER is tethered to an ERE, distinct classes of coregulatory proteins, including p160s coactivators, mediator complex proteins, and CAMP response element-bind-

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Abbreviations: AF, Activation function; AIB1, amplified in breast cancer-1 protein; AP-1, activator protein 1; C/EBPβ, CCAAT/enhancer-binding protein β; ChIP, chromatin immunoprecipitation; CREB, CAMP response element-binding protein; ER, estrogen receptor; ERE, estrogen response element; E2, estradiol; GRIP1, glucocorticoid receptor-interacting protein 1; HSF-2, heat-shock factor 2; LBD, ligand binding domain; NKG2E, killer cell lectin-like receptor; siRNA, small interfering RNA; SERM, selective estrogen receptor modulator; Sp1, stimulatory protein 1; SRC, steroid receptor coactivator; TK-luc, thymidine kinase promoter-luciferase gene; VERE, variant ERE-like element.

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activated by E\textsubscript{2} in all tissues, whereas alternative elements are activated by E\textsubscript{2} and SERMs in a tissue-specific manner and therefore can mediate tissue-specific effects of E\textsubscript{2} and SERMs (25–28).

Although the ERE and alternative elements are important mediators of the ER regulation of genes, recent microarray data from our laboratory and others (29–31) have revealed a pattern of gene regulation by E\textsubscript{2} and SERMs that cannot be understood by the pharmacology of the known response elements. For example, many genes regulated by E\textsubscript{2} with ER\textalpha and ER\textbeta are distinct from those regulated by ER\textbeta, and some genes that are regulated by tamoxifen are different from those regulated by raloxifene (30). Because it is known that both ER\textalpha and ER\textbeta activate an ERE (32, 33), it is likely that the genes differentially regulated by ER\textalpha and ER\textbeta contain response elements other than EREs. Furthermore, both tamoxifen and raloxifene activate AP-1 and Sp1 elements with ER\textalpha and ER\textbeta (27, 28, 34), making it unlikely that these elements are responsible for the differential activation of genes by these drugs. Together, these findings suggest that, in addition to the ERE and known alternative elements, other types of elements exist in ER target genes that are regulated by E\textsubscript{2} and SERMs. Identifying novel regulatory elements in ER target genes is a critical step in the elucidation of how estrogens and SERMs induce both therapeutic and adverse effects.

One of the ER target genes that exhibited an interesting pharmacological profile in our microarrays is the \textit{killer cell lectin-like receptor (NKG2E)} gene. In our studies with U2OS cells expressing ER\textalpha, both tamoxifen and raloxifene elicited an agonist effect similar to E\textsubscript{2} (30). These studies indicate that the NKG2E gene could be used as a model to better understand the mechanism whereby E\textsubscript{2} and SERMs activate regulatory elements in the context of a native gene rather than simple consensus elements such as AP-1 and Sp1. In the present study, we identified a complex ER regulatory element that requires the collaboration between ER\textalpha and multiple transcription factors. We also show that the p160 co-activators mediate the activation of the NKG2E gene by SERMs.

Materials and Methods

Preparation of stable cell lines

Tetracycline-inducible U2OS-ER\textalpha cells were prepared and maintained as described previously (30).

Plasmids, transfection, and luciferase assay

The NKG2E promoter was prepared by PCR using primers designed based on the human genome. The PCR product was cloned into the pGEM t-easy vector (Promega, Madison, WI) and sequenced. The promoter was then cloned upstream of the luciferase reporter gene. Deletions of the NKG2E promoter were prepared by using unique restriction sites (\textit{HindIII}, \textit{PstI}, and \textit{AclI}). Point mutations in the NKG2E promoter were prepared with the QuickChange Site-Directed Mutagenesis kit (Stratagene, La Jolla, CA). Transfections of the various vectors into wild-type U2OS cells were performed by electroporation (35). Oligonucleotides were cloned upstream of -32 to +45 thymidine kinase promoter-luciferase gene (TK-luc). Cells were assayed with a luciferase assay system according to the protocol of the manufacturer (Promega).

RNA extraction and quantitative real-time PCR

Total RNA was extracted and then treated with deoxyribonuclease using the Absolutely RNA miniprep kit (Stratagene). Reverse transcriptase reactions were performed using 1 µg of total RNA as described previously (36). Real-time PCR was performed with the Bio-Rad (Hercules, CA) iCycler using iQ SYBR Green Supermix. Mean \pm se was calculated using Prism curve-fitting program (version 3.03; GraphPad Software, San Diego, CA). Primers were designed using Oligo5.1 (National Biosciences, Inc., Plymouth, MN) and Amplify 1.2 (FaceWare) software and then blasted against all GenBank, Reference Sequence Nucleotides, European Molecular Biology Laboratory, DNA Data Bank of Japan, and Protein Data Bank sequences to verify that there was no cross-reaction with other genes. The sequences of the NKG2E primers used are as follows: forward, 5'-GCCAGCATTATCTCTCTTCAT-3'; reverse, 5’-AACATGATGAAACCCCGTCTAA-3’.

Chromatin immunoprecipitation (ChIP)

After treatments, cells were cross-linked, washed, collected, and lysed as described previously (37, 38). Fifty microliters of each sample were saved for total input. Immunoprecipitations were performed overnight at 4 C with anti-steroid receptor coactivator 1 (SRC-1) (1135), anti-heat-shock factor 2 (HSF-2) (32E) (Upstate, Charlottesville, VA), anti-ER\textalpha (HC-20), anti-c-jun (H-79), anti-C/EBP\beta (C-19) (Santa Cruz Biotechnology, Santa Cruz, CA), anti-glucocorticoid receptor-interacting protein 1 (GRIP1) (ab9261), and amplified in breast cancer 1 (AIB1) (ab2782) (Abcam, Cambridge, MA). DNA fragments were purified (QIAquick PCR Purification kit; Qiagen, Valencia, CA) and PCR amplified. The NKG2E chip primers used were as follows: forward, 5’-AGCCACCCCAAGTCTCTCAT-3’; reverse, 5’-TTCACTGGAGAGGTCAAGT-3’. Real-time PCR using primers spanning sites far from the NKG2E response element and non-immune assays served as negative controls (data not shown).

Coimmunoprecipitation

After treatments, U2OS-ER\textalpha cells were washed and lysed [150 mM NaCI, 1% Triton X-100, and 50 mM Tris (pH 7.5)]. Fifty microliters of each sample were saved for total input. Immunoprecipitations were performed overnight at 4 C with anti-HSF-2, anti-C/EBP\beta, or anti-c-jun antibodies mixed with protein-G agarose beads (Upstate). Beads were then washed and proteins eluted with SDS sample buffer containing dithiothreitol.

Immunoblotting

Proteins (15 µg) were separated using 4–12% gradient Bis-Tris gels (Invitrogen, Carlsbad, CA), transferred to polyvinylidene difluoride membranes, and detected with anti-HSF-2, anti-C/EBP\beta, anti-c-jun, or anti-ER\textalpha antibodies in blocking buffer and anti-IgG conjugated with horseradish peroxidase (BD PharMingen, San Diego, CA). An ECL detection system (GE Healthcare Biosciences, Piscataway, NJ) was used to visualize the proteins.

RNA interference

RNA interference for C/EBP\beta, c-jun, or nonspecific RNA was performed using SMARTpool small interfering RNA (siRNA) reagent (Dharmacon, Lafayette, CO). siRNA, 50 nM, was transfected into U2OS cells using oligofectamine (Invitrogen) according to the protocol of the manufacturer. For HSF-2, transient transfection of 3 µg \textit{pSilencer} 1.0-U6 (Ambion, Austin, TX) containing the hairpin forming oligonucleotides (5’-CAGGCGAGTACAACAGCATTCTCTCTTCATCC-3’ and 5’-CAGCGGACGATCACGAGCTTTCCATCTCTGTAAGTTGGTCT-3’) and an empty vector was used. At 72 h after transfections, cells were treated with E\textsubscript{2} for 12 h and collected for RNA and protein extraction.

EMSA

32P-labeled classical ERE or 32P-labeled NKG2E (-1852 to -1687) regulatory element was incubated with or without purified ER\textalpha (Invitrogen) in buffer (30 mM HEPES, 120 mM KCl, 2 mM EDTA, 10 mM

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MgCl, 24% glycerol, and 2 μg dIdC). Samples were then run on 6% polyacrylamide gel, which was dried and exposed to film.

Results

E2 and SERMs activate the NKG2E gene

U2OS-ERα cells were treated with E2, tamoxifen, and raloxifene for increasing times, and then NKG2E mRNA was measured by quantitative real-time PCR. All three drugs activated the NKG2E gene by 6 h, and the maximal activation was observed by 24 h (Fig. 1A). The E2-induced activation of the NKG2E gene was inhibited by tamoxifen and abolished by raloxifene (Fig. 1B). These results demonstrate that tamoxifen and raloxifene act as a mixed agonist/antagonist at the NKG2E gene, because they activate the gene in the absence of E2 and inhibit the gene in the presence of E2.

Multiple and distant elements are required for E2 and SERM activation of the NKG2E gene

To study the mechanism whereby E2 and SERMs regulate the NKG2E gene, we cloned a 3438 nucleotide fragment of the NKG2E promoter upstream of the luciferase reporter gene. The −3438 NKG2E promoter and several deletions (Fig. 2A) were transiently cotransfected into U2OS cells with an expression vector for ERα and then treated with E2, tamoxifen, or raloxifene. Consistent with the mRNA expression data, all three drugs stimulated the −3438 and −1825 NKG2E promoters. E2 produced a 12-fold activation of the NKG2E promoter, whereas tamoxifen and raloxifene activated the promoter by about 4- and 3-fold, respectively (Fig. 2B). The magnitude of activation of the NKG2E promoter by the SERMs is similar to their activation at AP-1 (26) and Sp1 (25) elements. No activation by E2 or SERMs occurred when the NKG2E promoter was deleted to −1686 (Fig. 2B). These results demonstrate that the ER regulatory element is located between −1825 and −1686.

To further map the ER response element, the −1825 to −1686 NKG2E promoter fragment was cloned upstream of minimal TK-luc, and then deletions at the proximal and distal ends were prepared (Fig. 2C). The constructs were transiently transfected into U2OS cells with the ERα expression vector. Similar to the native −3438 NKG2E promoter (Fig. 2B), the −1825 to −1686 fragment was activated by both E2 and SERMs (Fig. 2D). Surprisingly, the activation was abolished by deletions at either end of the −1825 to −1686 region (Fig. 2D). These findings demonstrate that E2 and SERM activation of the NKG2E regulatory region requires multiple and distant elements.

The activation of the NKG2E promoter by E2 and SERMs requires a transcriptional factor cluster and a unique variant ERE

The −1825 to −1686 region contains potential binding sites for multiple transcription factors, including AP-1, myeloid zinc finger (MZF-1), HSF-2, C/EBPβ, GATA, and octamer-binding transcription factor 1. This region also contains a variant ERE-like element (vERE) (Fig. 3A). These elements were individually mutated and then tested in transient transfections for responses to E2 and SERMs. The activation by E2 was markedly inhibited by mutations in the AP-1, HSF-2, and C/EBPβ elements (Fig. 3B). These mutations also abolished tamoxifen and raloxifene activation of the promoter (Fig. 3B). Mutations in the other transcription factor elements (MZF-1, GATA, and octamer-binding transcription factor 1) did not have any effect on the activation by E2 or SERMs (data not shown). The vERE (5′ GGTAACC TGACC 3′) has a perfect distal half-site and a proximal half-site with one nucleotide difference compared with the classical ERE (5′ GGTCAnnnTGACC 3′). This site also differs from the classical ERE in that it contains two nucleotides, rather than the usual three, between the half-sites (Fig. 3C, top). Mutations in the proximal or the distal vERE half-site sequence (Fig. 3C, middle and bottom, respectively) abolished the activation by E2 and SERMs (Fig. 3D), demonstrating that the complete vERE is required for activation.

To explore the functional significance of the vERE, we cloned the vERE upstream of TK-luc (Fig. 4A) and transfected the construct into U2OS cells along with an ERα expression vector. We found that the vERE by itself was not activated by E2 (Fig. 4B) or SERMs (data not shown). Similarly, the transcription factor cluster containing the AP-1, HSF-2, and C/EBPβ elements upstream of TK-luc was not regulated by E2 (Fig. 4B) or SERMs (data not shown), although it contains an AP-1 element. To determine whether the vERE is influenced by cluster proteins, an oligonucleotide containing the cluster elements positioned 24 bp proximal to the vERE was cloned upstream of TK-luc (Fig. 4A, bottom). The activation by E2 was restored when the cluster elements were positioned adjacent to the vERE (Fig. 4B). However, the
ERα interacts with AP-1, HSF-2, and C/EBPβ

The observation that mutations in the binding sites for the cluster proteins markedly inhibited E2 and SERM activation of the NKG2E gene demonstrates the importance of these elements in NKG2E regulation. However, it is important to determine whether ERα interacts with the cluster proteins. U2OS-ERα cells were treated for increasing periods of time with E2, and cellular lysates were immunoprecipitated with antibodies to c-jun, HSF-2, or C/EBPβ. The immunoprecipitated proteins were run on a SDS-polyacrylamide gel, and Western blots were done with an ERα antibody. ERα interacted strongly with HSF-2 and C/EBPβ after 1 h of E2 treatment (Fig. 5A). A decrease in the amount of HSF-2 and C/EBPβ was observed at 2 and 3 h. This decrease might be attributable to the recruitment of ER, coactivators, and possibly other factors that make the antibodies less accessible for binding to HSF-2 and C/EBPβ. ERα also interacted with c-jun, but the binding was weaker at all time points. These results demonstrate that ERα interacts with a transcription factor complex containing c-jun, HSF-2, and C/EBPβ.

Kinetics of ERα and cluster protein assembly on the NKG2E promoter after E2 treatment

Whereas the coimmunoprecipitation studies demonstrate that ERα interacts with the cluster proteins in intact cells, they do not show that these factors interact at the NKG2E promoter. To assess the occupancy of ERα and cluster proteins at the NKG2E promoter over time, we performed ki-
ChIP assays. U2OS-ERα cells were treated with E2 for 15, 30, 60, or 180 min before they were cross-linked with formaldehyde. ERα binding to the NKG2E promoter was first detected 15 min after E2 treatment and steadily increased to reach maximal levels by 180 min (Fig. 5B). The kinetics of HSF-2 binding to the NKG2E promoter was similar to ERα. Significant C/EBPβ binding only occurred at 180 min (Fig. 5B). Similarly, recruitment of c-jun to the NKG2E promoter was only observed at 180-min treatment with E2, but the basal levels of c-jun bound to NKG2E gene were high (data not shown), suggesting that c-jun is already present at the promoter before E2 treatment.

Silencing of c-jun, HSF-2, and C/EBPβ inhibits E2-mediated activation of the NKG2E gene

To further explore a functional role for cluster proteins in ERα activation of the NKG2E gene, we used RNA interference to silence the cluster protein genes. After 3 days of gene silencing, we observed a significant reduction in c-jun, HSF-2, and C/EBPβ protein levels (Fig. 5C). The decline in protein levels was associated with a 40–70% reduction in the activation of the NKG2E gene by E2 (Fig. 5D–F) compared with cells treated with control siRNA. Silencing treatments did not affect gene expression of control genes regulated by E2 (naked cuticle, solute carrier family 4, H19, and cat eye syndrome chromosome region, candidate 6) (data not shown). These results provide additional evidence that the amount of cluster proteins present in cells determines the magnitude of ER activation of the NKG2E gene.

The activation of the NKG2E promoter by E2 and SERMs requires both activation function 1 (AF-1) and AF-2 domains of ERα

ER contains two functional domains, AF-1 and AF-2, that mediate transcriptional activation in response to ligands (40). AF-1 is located in the N-terminal A/B domains and is constitutively active (41). The AF-1 is activated by antiestrogens, such as tamoxifen (26), which causes the recruitment of coactivators (42–44). AF-2 is located in the ligand binding domain (LBD) and is activated by agonists that lead to the recruitment of coactivators (42–44). AF-2 is located in the ligand binding domain (LBD) and is activated by agonists that lead to the recruitment of coactivators (42–44). AF-2 is located in the ligand binding domain (LBD) and is activated by agonists that lead to the recruitment of coactivators (42–44). AF-2 is located in the ligand binding domain (LBD) and is activated by agonists that lead to the recruitment of coactivators (42–44). AF-2 is located in the ligand binding domain (LBD) and is activated by agonists that lead to the recruitment of coactivators (42–44). AF-2 is located in the ligand binding domain (LBD) and is activated by agonists that lead to the recruitment of coactivators (42–44). AF-2 is located in the ligand binding domain (LBD) and is activated by agonists that lead to the recruitment of coactivators (42–44).
whereas the effect of the AF-2 mutants was not as pronounced in the presence of raloxifene. These results indicate that E2 and SERMs promote AF-1 and AF-2 cooperation to activate the NKG2E promoter.

Coactivator-specific recruitment by E₂ and SERMs

As mentioned above, we used the NKG2E gene as a model to better understand the mechanism whereby target genes are regulated by E₂ and SERMs. Therefore, we next sought to study the downstream factors involved in this regulation. It is well known that E₂ activates genes by recruiting p160/SRC coactivators, SRC-1, SRC-2 (GRIP1), and SRC-3 (AIB1), (10, 46) to the AF-2 surface of ER (47–49). However, the mechanisms involved in SERM activation of genes are poorly understood. After identifying the ER regulatory unit in the NKG2E gene, we examined whether the p160 coactivators play a role in the activation by SERMs. We performed ChIP with ERα or p160s antibodies. ERα was recruited to the NKG2E element after treatment with all three drugs (Fig. 6B). E₂ and tamoxifen recruited GRIP1 (Fig. 6D) and AIB1 (Fig. 6E) to the NKG2E gene. Raloxifene recruited only AIB1 to the NKG2E gene (Fig. 6E). None of the drugs recruited SRC-1 to the NKG2E gene (Fig. 6C). It is unlikely that the lack of recruitment of SRC-1 is attributable to the differential expression of coactivators, because our microarray data show that SRC-1 and AIB1 mRNA levels are 7.5-fold and 2-fold higher than GRIP1 mRNA in U20S-ERα cells (data not shown). Furthermore, we found that SRC-1 is recruited to other genes regulated by E₂ (data not shown), demonstrating that SRC-1 protein is expressed in the U2OS cells. These results demonstrate that level of recruitment of GRIP1 correlated with the activity of the ligands in functional assays and provide evidence that coactivators mediate the activation of the NKG2E gene by both E₂ and SERMs.

Discussion

We studied the NKG2E gene as a model to characterize a regulatory element derived from a native gene that is activated by both E₂ and SERMs in the presence of ERα (30). The ER regulatory element was mapped to the −1.83 to −1.68 kb region of the NKG2E promoter. Surprisingly, our deletion and mutation studies showed that the NKG2E regulatory element is extremely complex and requires multiple and distant elements for regulation by E₂ and SERMs. The activation of the NKG2E promoter by E₂ and SERMs was inhibited by mutations in c-jun, HSF-2, and C/EBPβ elements.
The NKG2E promoter does not contain a classical ERE but instead it has a vERE. This sequence includes a variant proximal half-site and a perfect distal half-site separated by only two nucleotides instead of three, which is characteristic of the classical EREs. Mutations in both half-sites of the vERE blocked the activation by E2 and SERMs, demonstrating that both sites are required for full activation. This vERE was not detected in software designed to identify EREs. Furthermore, ERα did not bind to this element in gel shift assays, and the element was not activated by E2 or SERMs upstream of the TK promoter. These findings demonstrate that the vERE is inactive on its own. In contrast, the vERE was activated by E2 and SERMs in the presence of a transcription factor cluster containing c-jun, HSF-2, and C/EBPβ elements positioned adjacent to the vERE. Surprisingly, the cluster by itself was not regulated upstream of TK, although it contains an AP-1 element.

Our ChIP analyses confirm that E2 causes the assembly of a complex at the NKG2E regulatory element that consists of ERα, c-jun, HSF-2, and C/EBPβ. The complex interactions between ERα and multiple transcription factors that are required for binding to the vERE could explain our inability to observe ERα binding in nuclear extracts prepared from U2OS-ERα cells treated with E2 (data not shown). We propose that the role of the transcription factor cluster is to create a platform for ER binding and a functional ERE by stabilizing ER binding to the vERE. Without the cluster proteins, any binding of ERα to the vERE is probably too unstable for it to remain there long enough to recruit coactivators and other factors required for gene activation. It is known that single transcription factors, such as AP-1 (26) and Sp1 (18), can interact with ER to alter the magnitude of regulation by estrogens and SERMs. More recently, it has been shown that the transcription factor forkhead box A1 can be essential for ER binding to DNA regulatory elements (50, 51). Our study extends these findings by showing that ERα can interact with a cluster of transcription factors to regulate transcription at a vERE that by itself is incapable of binding ERα and therefore is nonfunctional. Unlike the typical EREs in known target genes, which ERα can bind to independent of other factors, our findings indicate that there are EREs induced by transcription factors. ERα can activate inducible EREs, such as the one in the NKG2E promoter, only when it is tethered to a transcription factor complex. The activation of the vERE...
most likely results from direct ER/DNA binding as demonstrated by the observation that the ERE binding mutant (HE82) was ineffective at activating the NKG2E promoter. The observation that a vERE can be activated, although the half-sites are separated by only two nucleotides instead of the standard three, might significantly expand the number of EREs in the genome. Using bioinformatics to search the human genome, we found that there are 8199 genes that contain a putative ERE with a two nucleotide spacer. Of these genes, 3307 have expression data, of which 21 genes were regulated by estrogens by at least 1.5-fold change. Whereas it is unknown whether any of these genes are regulated by EREs containing a two nucleotide spacer, our data with the NKG2E regulatory element indicates that it conceivable that some of these genes are regulated by two spacer EREs.

We demonstrated that silencing of c-jun, HSF-2, and C/EBP genes with siRNA resulted in a marked decline in the magnitude of induction of NKG2E mRNA after treatment with E2. These findings suggest that, in cells containing ERα, the magnitude of activation of the NKG2E gene will be altered by the relative levels of c-jun, HSF-2, and C/EBP. In this model, only those tissues that express sufficient levels of c-jun, HSF-2, and C/EBP would be expected to show an activation of the NKG2E gene by E2 and SERMs with ERα. Interestingly, a similar regulatory unit is also present in the NKG2D family member, which is differentially expressed in tissues. However, like NKG2E, NKG2D is also activated by E2 and SERMs in the U2OS cells (data not shown). Our data along with the observations that HSF-2 (52) or C/EBPβ (53) knock-out mice exhibit female subfertility or sterility also suggest that these transcription factors may have a more extensive role in the tissue-specific regulation of target genes by estrogens.

It is well established that SERMs exert antagonistic effects on gene expression by disrupting the AF-2 surface and recruiting corepressors, such as nuclear receptor corepressor, to ERs. In contrast, the mechanism underlying the agonist action of SERMs that leads to gene activation is poorly understood. Studies by Kushner et al. (19) have suggested that the activation at AP-1 elements by SERMs may involve the sequestration of corepressors, such as nuclear receptor corepressor or silencing mediator of retinoic acid and thyroid hormone receptors. By blocking corepressor recruitment to ER, the SERMs would allow activation function-1 (AF-1) activity in the A/B domain to operate more efficiently so as to activate the gene (49). The role of the p160 coactivators in SERM gene activation is unclear. Structural analysis of the ER LBDs has shown that SERMs act as antagonists by blocking the movement of helix 12 to a position that creates a functional AF-2 surface. In fact, in the presence of SERMs, helix 12 actually occupies the coactivator binding site (49). This prevents the binding of coactivators to the ER LBD required to activate gene transcription. These findings suggest that SERMs do not recruit p160s to activate genes. However, several studies suggest that coactivators may be involved in gene activation in response to SERMs. Coactivators can bind
to the A/B domain of ER in the presence of SERMs (42–44), which could potentiate intrinsic AF-1 activity. Shang and Brown (54) reported that tamoxifen recruits the coactivator SRC-1 to genes activated in endometrial cells but not in MCF-7 breast cancer cells. Alternatively, it is conceivable that the AF-2 surface is not destroyed when ER is tethered to transcriptional factor complexes bound to NKG2E gene, which would allow some coactivator recruitment to the AF-2 surface even in the presence of SERMs. Another possible explanation is that the SERMs recruit coactivators to a unique surface of ERs. Regardless of the precise location of the coactivator binding to ERα, our studies clearly indicate that the agonist action of SERMs at the NKG2E gene is mediated by ERα recruitment of p160s coactivators.

Our study demonstrates that the collaboration between multiple transcription factors and ER could be an important mechanism to elicit tissue-specific effects and determine the magnitude of gene activation by E2 and SERMs. A greater understanding of the elements in native genes that mediate the regulation by E2 and SERMs could provide the basis to develop safer SERMs that regulate only selective genes in target tissues.

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Address all correspondence and requests for reprints to: Dale Leit-man, Department of Obstetrics, Gynecology, and Reproductive Sciences, 513 Parnassus Avenue, S-1258, San Francisco, California 94143. E-mail: leitman@obgyn.ucsf.edu.

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