IKKe has recently been identified as a breast cancer oncogene. Elevated levels of IKKe are associated with cell survival and growth. Here, we show that IKKe interacts with and phosphorylates estrogen receptor α (ERα) on serine 167 in vitro and in vivo. As a result, IKKe induces ERα transactivation activity and enhances ERα binding to DNA. Cyclin D1, a major target of ERα, is transcriptionally up-regulated by IKKe in a phospho-ERα-Ser-167-dependent manner. Further, overexpression of IKKe induces tamoxifen resistance, whereas knockdown of IKKe sensitizes cells to tamoxifen-induced cell death. These data suggest that ERα is a bona fide substrate of IKKe and IKKe plays an important role in tamoxifen resistance. Thus, IKKe represents a critical therapeutic target in breast cancer.

Breast carcinoma is the most common cancer among women in developed countries, and about 70% of these tumors express estrogen receptor (ER)α. ERα-positive tumors are associated with a well differentiated phenotype and have a better prognosis than ERα-negative tumors (1). The major reason is that ERα-positive tumors initially respond well to anti-estrogen agents such as tamoxifen. However, a significant portion of ERα-positive tumors eventually become resistant to anti-estrogen therapy (2–4). The underlying molecular mechanisms have been linked to loss of ERα expression caused by DNA hypermethylation and deregulation of certain microRNAs (2, 5). It is noted that a number of patients with tamoxifen-resistant breast cancer remain ERα-positive (2, 4). Growing evidence shows that ERα membrane-initiated steroid signaling activities and cross-talk with growth factor signal transduction pathways may contribute to tamoxifen resistance. Activation of ERα outside the nucleus leads to the activation of surface tyrosine kinase receptors (e.g. IGF-IR, EGFR, and HER2) as well as interaction with cellular kinases and adaptor molecules (e.g. c-Src or the p85α regulatory subunit of phosphatidylinositol-3-OH kinase), which in turn lead to the activation of mitogen-activated protein kinase (MAPK) and AKT pathways known to enhance cell proliferation and survival (6–8). The activation of these signaling pathways causes phosphorylation of ERα and/or its co-activators and co-repressors, thereby increasing nuclear ERα activity (4, 9).

IKKe is a member of IκB kinase (IKK) family and activates NFκB through phosphorylation and degradation of IκB (10–12). It is primarily activated by interferon (IFN), PMA, and activation of the T-cell receptor (13, 14). Using complementary genetic approaches, Boehm et al. (15, 16) identified IKKe as a breast cancer oncogene, which is frequently amplified/overexpressed in human breast cancer. Recently, we have shown frequent alterations of IKKe in ovarian cancer (17). In this report, we demonstrate that IKKe phosphorylates Ser-167 of ERα. IKKe induction of ERα transactivation and cyclin D1 expression is dependent on phosphorylation of Ser-167. Knockdown of IKKe sensitizes breast cancer cells to tamoxifen-induced cell death and growth arrest, whereas ectopic expression of IKKe exhibits the opposite effect. These data indicate that IKKe plays an important role in regulation of ERα activity and tamoxifen resistance, and thus could be a therapeutic target in breast cancer.

**EXPERIMENTAL PROCEDURES**

**Cells and Reagents**—All breast cancer cell lines used in this study were purchased from ATCC, maintained in DMEM with 10% fetal bovine serum. MCF10A was maintained in MACo5 with 10% FBS. Cells, when used for ERα phosphorylation, tamoxifen treatment, and ERα transactivation activity experiments, were cultured in phenol red-free DMEM with charcoal-stripped serum. The IKKe antibody (I-4907) was obtained from Sigma. CCND1 (sc-8396), ERα (sc-543), and p-ERα-Ser-167 (sc-101676) antibodies were purchased from Santa Cruz Biotechnology. Recombinant ERα and IKKe were obtained from Thermo Scientific and Cell Signaling, respectively.

**Plasmids, Transfection, and Infection**—The pCMV-Myc tagged IKKe was described previously (17). Dominant negative mutant IKKe-K38A was created with the QuickChange site-directed mutagenesis kit (Stratagene) using pCMV-Myc-IKKε as a template. The pLKO1-shRNAs of IKKe and GFP were acquired from Open Biosystems. The pEGFP-ERα, pCMV-Myc-ERα, and pCMV5-Myc-ERα-S167A were obtained by PCR amplification using pCMV5-ERα and pCMV5-ERα-S167A (kindly provided by Dr. Benita S. Katzenellenbogen) as templates, and the PCR products were cloned to pEGFP (Clontech) at BglII/EcoRI and pCMV-Tag3B (Stratagene) at BamHI/EcoRI.

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IKKe Phosphorylates ERα and Induces Tamoxifen Resistance

IKKe phosphorylates ERα and induces tamoxifen resistance. The GST-ERα, GST-ERα-S167A (amino acids 140–180), ERE-Luc, cyclin D1-Luc, and β-galactosidase plasmids were described previously (18, 19).

Lipofectamine 2000 (Invitrogen) was used for the transfection experiments with indicated plasmids in the figure legend. Stable knockdown of IKKe was carried out in T47D cells that were infected with lentivirus pLKO1-Ikke-shRNA clone 3 (sense 5′-TGGGCGAGGCTAATGTTTCCG-3′ and antisense 5′-CGAAAATTCAGCTCTGCCCC-3′) and subsequently selected with puromycin. The cells were also infected with pLKO1-GFP-shRNA virus, which has been commonly used as a control in pLKO1-shRNA system (15, 20). In addition, siRNA-Ikke was also used to knockdown IKKe in T47D cells. The sequence for siRNA-Ikke is 5′-AAGGCCGCGAGAAGAGUGCAGT-3′ and 3′-dTdTCCGGCCAGCUCUGACUCAGC-5′. Mismatched (scramble) siRNA-Ikke was obtained by change of 3 bases (underline): 5′-AAGGCCGCGAGAAGAGUGCAGT-3′ as previously described (18). ChIP assay was performed using a scaffold (CBS). Western blot analysis of indicated cell lines expressed transfected plasmids. Actin was used as a loading control (bottom). E and F, endogenous ERα binds to IKKe, T47D cells, expressing ERα and IKKα (panels 3 and 4), were immunoprecipitated with anti-ERα and detected with anti-iKKα antibody (E) and vice versa (F; panels 1 and 2). IgG was used as a control for immunoprecipitation, and actin is a loading control (bottom)

In vitro Kinase Assay, in vivo [32P]Pi, Cell Labeling, and Mass Spectrometry—In vitro IKKe kinase assay was performed as described previously (11, 17). Briefly, the reaction was carried out in the presence of 10 μCi of [γ-32P]ATP (NEN) and 3 μM cold ATP in 30 μl of buffer containing 20 mM Hepes (pH 7.6), 10 mM MgCl2, 50 mM NaCl, 0.1 mM sodium vana-
date, 20 mM β-glycerolphosphate, and 1 mM DTT using GST-ERα as substrate. After incubation at 30 °C for 30 min, the reaction was stopped by adding protein-loading buffer; the proteins were then separated in SDS-PAGE gels. Each experiment was repeated three times. The relative amounts of incorporated radioactivity were determined by autoradiography and quantitated with a PhosphorImager (Molecular Dynamics).

For in vivo labeling, MCF10A cells were transfected with ERα/Myc-IKKα. After serum starvation overnight, cells were labeled with [32P]Pi (0.5 mCi/ml) in phenol red-free MEM without phosphate for 4 h. ERα was immunoprecipitated and separated by SDS-PAGE prior to transferring to membrane for detection and quantification of phosphorylated ERα.

Mass spectrometry was used to map IKKe phosphorylation site of ERα. After separation of in vitro IKKe/ERα kinase reactions in SDS-PAGE, ERα bands were excised and washed. Proteins were reduced with Tris(carboxyethyl)phosphine and alkylated with iodoacetamide. Samples were digested overnight with modified sequencing grade trypsin (Promega, Madison, WI). Peptides were extracted and concentrated under vacuum centrifugation. A nanoflow liquid chromatograph (U3000, LC Packings/Dionex, Sunnyvale, CA) coupled to an electrospray hybrid ion trap mass spectrometer (LTQ Orbitrap, Thermo, San Jose, CA) was used for tandem mass spectrometry peptide sequencing. Peptides were separated on a C18 reverse phase column (LC Packings C18 Pepmap, 75 μm ID × 15 cm) using a 40-min gradient from 5% B to 50% B (A: 2% acetonitrile/0.1% formic acid; B: 90% acetonitrile/0.1% formic acid). The flow rate on the analytical column was 300 nl/min. Five tandem mass spectra were acquired for each MS scan using 60 s exclusion for previously sampled peptide peaks. Sequences were assigned using Mascot data base searches. Oxidized methionine, deamidation, carbamidomethyl cysteine, and phosphorylated serine, threonine, and tyrosine were selected as phosphorylated serine, threonine, and tyrosine were selected as inspection of the tandem mass spectra and coalesced into

Western Blot, Co-immunoprecipitation (co-IP), Immunofluorescence, Luciferase Reporter Assay, Chromatin Immunoprecipitation (ChiP), and RT-PCR—Western blots, co-IP, immunofluorescence, and luciferase reporter assays were performed as previously described (18). ChiP assay was performed using a kit (Upstate) following the manufacturer’s instruction. The
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primers used for CCND1 were as follows: forward (−1039) (AACAAAACCAATTAGGAACCTT), reverse (−770) ATTTCTCTCAGTTCTGTCTCT.

Reverse transcription-PCR (RT-PCR) was performed as previously described (17). The primers were CCND1: 5′-GAA- CAGAAGTGCGAGAAGGAG-3′ (forward), 5′-AGGCGGT- AGTGGCAGAGGA-3′ (reverse) and GAPDH: 5′-CATG- TTGTCATGGTGTAACCA-3′ (forward), and 5′-AGT- GATGGCACTGTGGTAT-3′ (reverse).

Cell Survival, Focus Formation, and Apoptosis Assays—Cell survival was examined by MTT assay as previously described (17). Briefly, cells were diverted into 96-well plates with 1 × 10⁴ cells/well. Following a 24-h incubation, cells were treated with different concentrations of tamoxifen for 72 h and then assayed for total cell viability (MTT assay). For focus formation, cells were released from culture flasks with Trypsin-EDTA (Invitrogen) and resuspended at a concentration of 100 cells/ml and placed in a 6-well plate. The cells were allowed to grow for 7 days in the presence or the absence of tamoxifen at which point they were washed with phosphate-buffered saline (pH 7.4) and stained with 1 mg/ml p-iodonitrotetrazolium violet in DMEM (21). Apoptosis was analyzed with Annexin V-fluorescein isothiocyanate apoptosis detection kit following the manufacturer’s instruction (BD Pharmingen).

Statistical Analysis—For luciferase activity, cell survival, and apoptosis, the experiments were repeated at least three times in triplicate. The data are represented by means ± S.D. Differences between control and testing cells were evaluated by Student’s t test.

RESULTS

IKKε Phosphorylates and Interacts with ERα—Previous studies have shown that phosphorylation of ERα by serine/threonine protein kinases (e.g. Erk, casein kinase II, pp90ε1k, Akt, and IKKα) induces tamoxifen resistance (9,22–26). Frequent alterations of IKKε kinase in breast cancer (15, 16) prompted us to examine whether IKKε regulates ERα. As an initial step, we examined IKKε expression in a panel of breast cancer cell lines. Consistent with a previous report (15), expression of IKKε has no correlation with ERα status in breast cancer cell lines (Fig. 1A). Further, in vitro kinase assay was performed by incubation of recombinant IKKε and full-length human recombinant ERα. Fig. 1B shows that IKKε strongly phosphorylated ERα in vitro. To demonstrate that IKKε phosphorylates ERα in vivo, MCF10A, an ERα-negative cell line, was transfected with ERα and ERα/Myc-IKKε. After ³²P orthophosphate labeling, ERα was immunoprecipitated with anti-ERα antibody. The immunoprecipitates were
These findings indicate that ER location of the phosphorylation site as serine 167. The phosphorylated ER was transfected with ERE-Luc, and performed (Fig. 2A). phosphorylation of Ser-167 is required for IKKβ in vivo. Furthermore, endogenous ERα and IKKe were able to form a complex in T47D cells (Fig. 1, E and F), which expresses ERα as well as high levels of IKKe (Fig. 1A). These findings indicate that ERα is a substrate of IKKe.

**IKKe Phosphorylates ERα at Serine 167**—To define the site in ERα that is phosphorylated by IKKe, in vitro IKKe kinase assay was carried out using GST fusion proteins containing different portions of ERα as substrates. A potential phosphorylation site(s) was mapped to the amino acid 90–324 region of ERα (Fig. 2A). Mass spectrometry analysis revealed serine 167 (Ser-167) as a possible phosphorylation site (Fig. 2B). To verify if ERα-Ser-167 is phosphorylated by IKKe, we created non-phosphorylatable ERα-S167A by converting Ser-167 into alanine and performed in vitro IKKe kinase assay using the wild-type and S167A mutant GST-ERα as substrates. Fig. 2C shows that wild-type GST-ERα but not GST-ERα-S167A is phosphorylated by IKKe. Furthermore, in vivo [32P]orthophosphate labeling was performed in MCF10A cells, which were transfected with either ERα or ERα-S167A together with and without Myc-IKKe. We observed IKKe phosphorylation of wild-type but not S167A mutant ERα (Fig. 2D).

In addition, immunoblotting was performed with anti-pERα-Ser-167 antibody in MCF10A cells transfected with ERα together with wild-type and dominant-negative (DN) IKKe. As shown in Fig. 2E, IKKe, but not DN-IKKe, phosphorylates ERα-Ser-167. To further demonstrate that ERα-Ser-167 is phosphorylated by IKKe, we ectopically expressed IKKe in MCF7 cells and then immunoblotted and immunostained with anti-pERα-Ser-167 antibody. Fig. 2, F and G show elevated pERα-Ser-167 levels in IKKe-transfected cells when compared with pCMV vector-treated cells.

Moreover, knockdown of IKKe decreases pERα-Ser-167 in T47D cells (Fig. 6A), which express both ERα and high level of IKKe (Fig. 1A). Based on these results, we conclude that IKKe phosphorylates Ser-167 of ERα in vitro and in vivo.

**IKKe Induces ERα Transactivation Activity through Phosphorylation of Ser-167**—Previous studies have demonstrated that the ERα-Ser-167 is one of major phosphorylation sites to activate ERα (6, 26). Therefore, we further assessed whether IKKe induces ERα transactivation activity and, if present, whether the activation depends on phosphorylation of Ser-167. Reporter assay was performed in MCF7 cells transfected with estrogen response element promoter-luciferase (ERE-Luc) reporter together with and without IKKe. Fig. 3A shows that ERE-Luc activity was induced by ectopic expression of wild type but not DN-IKKe, implying that IKKe activation of ERα is kinase-dependent. Further, we transfected MCF10A cells with wild-type ERα and non-phosphorylatable ERα-S167A together with and without IKKe. Expression of IKKe alone did not induce ERE-Luc activity in MCF10A cells. However, co-expression of ERα/IKKe but not ERα-S167A/IKKe significantly stimulated the reporter activity (Fig. 3B), indicating that IKKe activates ERα through phosphorylation of Ser-167.

**IKKe Up-regulates Cyclin D1 Expression Primarily through Phospho-ERα-Ser-167**—Because cyclin D1 (CCND1) is a major target of ERα (27), we next investigated whether cyclin D1 is separated on SDS-PAGE and exposed on the film. Fig. 1C displays that ectopic expression of IKKe induces ERα phosphorylation in vivo.

We next examined whether IKKe interacts with ERα. The communoprecipitation was performed in MCF10A cells, which had been transfected with GFP-ERα and Myc-IKKe. ERα was readily detected in Myc-IKKe immunoprecipitates and vice versa (Fig. 1D). Furthermore, endogenous ERα and IKKe were able to form a complex in T47D cells (Fig. 1, E and F), which expresses ERα as well as high levels of IKKe (Fig. 1A). These findings indicate that ERα is a substrate of IKKe.

**FIGURE 3. IkkE induces ERα transactivation activity through phosphorylation of Ser-167.** A, IKKe activates ERα in a kinase-dependent manner. MCF7 cells were transfected with ERE-Luc together with wild-type and dominant-negative IKKe as well as β-galactosidase. Following 48 h of incubation, luciferase activity was measured and normalized to β-galactosidase. Results are the mean ± S.E. of three independent experiments performed in triplicate. B, phosphorylation of Ser-167 is required for IKKe-induced ERα activation. MCF10A cells were transfected with ERE-Luc, β-galactosidase, and other indicated plasmids. Luciferase assay was performed as described above. Asterisks indicate p < 0.05.

**FIGURE 2. IKKe phosphorylates ERα-Ser-167.** A, IKKe phosphorylates N-terminal region (amino acids 90–324) of ERα. In vitro IKKe kinase assay was performed using different truncated GST-ERα fusion proteins as substrates (top). Coomassie Blue staining shows GST-ERα fusion proteins used in the kinase assay (bottom). B, tandem mass spectrum analysis. The inset shows the peptide measurement in the survey scan. Arrows indicate the fragment ions that confirm the location of the phosphorylation site as serine 167. The m/z value of the y13 ion reflects that the phosphorylation is not present in that fragment; the m/z values b3 and y14 (2+) indicate the presence of the phosphorylation. The Mascot score for the phosphopeptide was 59 (cutoff score is usually 20–25 for reliable data); in addition, peptides with one or two methionine oxidations were also observed with Mascot scores of 37 and 51, respectively. The mass measurements of the oxidized forms were accurate to 4.2 ppm and 2.6 ppm. C, IKKe phosphorylates ERα-Ser-167 in vitro. In vitro IKKe kinase assay was performed using GST-WT- and -S167A-ERα as substrates (top). The bottom panel is Coomassie Blue staining. D, IKKe phosphorylates ERα-Ser-167 in vivo. MCF10A cells were transfected with wild-type and S167A mutant ERα together with and without IKKe. In vivo labeling was performed as described in Fig. 1C (top). Panels 2 and 3 show expression of transfected plasmids. E, anti-pERα-Ser-167 antibody detects IKKe-induced ERα phosphorylation. MCF10A cells were transfected with Myc-ERα and Myc-IKKe. After 48 h of incubation, cells were lysed and immunoblotted with anti-pERα-Ser-167 (top) or -ERα (panel 2). Myc (panel 3), and -actin (bottom) antibodies. F and G, IKKe phosphorylates endogenous ERα-Ser-167. MCF7 cells were transfected with IKKe or pCMV vector and immunoblotted (F) or immunofluorescence stained (left panel of G) with indicated antibodies. Middle and right panels of G are DAPI staining and merged image, respectively.
regulated by IKKε, and, if this is the case, whether IKKε-regulated cyclin D1 depends on pERα-Ser-167. MCF7 (ERα-positive) and MCF10A (ERα-negative) cells were co-transfected with IKKε/ERα or IKKε/ERα-S167A. As shown in Fig. 4, A and B, ectopic expression of IKKε up-regulates cyclin D1 more significantly in MCF7 than MCF10A cells. Further, co-expression
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of IKKε with ERα, but not ERα-S167A, induces CCND1 in MCF10A cells (Fig. 4B). However, ectopic expression of IKKε alone also induced cyclin D1 mRNA and protein levels (lane 2 of Fig. 4B), suggesting that IKKε regulation of cyclin D1 could also be mediated by other pathway(s).

We next performed the luciferase reporter assay in MCF10A cells that were transfected with pGL3-cyclin D1-Luc, IKKε, and ERα or ERα-S167A. As shown in Fig. 4C, co-expression of IKKε and ERα significantly induced cyclin D1 promoter activity. Moreover, IKKε failed to activate cyclin D1 promoter in cells transfected with IKKε-S167A (Fig. 4C). In addition, ChIP assay revealed that expression of IKKε enhanced capability of ERα but not ERα-S167A binding to cyclin D1 promoter (Fig. 4D). Taken collectively, we concluded that IKKε up-regulates cyclin D1 primarily through phosphorylation of ERα-Ser-167.

IKKε Plays an Important Role in Tamoxifen Sensitivity—Having demonstrated IKKε phosphorylation of ERα on Ser-167, a site which has been shown to be involved in tamoxifen resistance (6, 18, 24), we subsequently examined the role of IKKε in tamoxifen sensitivity. Of ERα-positive cell lines, MCF7 expressed relatively lower levels of IKKε than T47D and MDA-MB-361 (Fig. 1A). Thus, we ectopically expressed IKKε in MCF7 (Fig. 5A). Following tamoxifen treatment, MTT and Annexin V/FACS assays revealed that total cell death and apoptosis were significantly reduced in IKKε-transfected MCF7 cells compared with the cells transfected with vector alone (Fig. 5, B and C). Moreover, tamoxifen-induced inhibition of focus formation was reduced in MCF7 cells overexpressing IKKε (Fig. 5D).

To further demonstrate the role of IKKε in tamoxifen sensitivity, we knocked down IKKε in T47D cells by either infection of lentiviruses/shRNA-IKKε or transfection of siRNA-IKKε. The cells infected/transfected with shRNA-GFP and scramble/mismatched siRNA-IKKε were used as controls. Western blot analysis shows that IKKε was efficiently knocked down by both shRNA-IKKε and siRNA-IKKε as compared with controls (Fig. 6A). Accordingly, pERα-Ser-167 levels were reduced by depletion of IKKε (Fig. 6A). Further, the knockdown of IKKε by shRNA or siRNA increased tamoxifen-induced total cell death and apoptosis at 10 μM (Fig. 6B and C; p < 0.05). Tamoxifen-inhibited focus formation was also enhanced by knockdown of IKKε (Fig. 6D). In addition, we noticed that parental T47D cell is less sensitive to tamoxifen than MCF7 (Figs. 5, B–D versus 6, B–D) that could be attributed to elevated level of IKKε in T47D (Fig. 1A).

Furthermore, we have selected 18 ERα-positive primary breast cancers, 8 of which were tamoxifen-resistant, and examined IKKε and pERα-Ser-167 levels. Immunoblotting analysis revealed high levels of IKKε and pERα-Ser-167 in 5 tamoxifen-resistant tumors but not the rest of the specimens (Fig. 6E and data not shown), further suggesting that IKKε plays an important role in phosphorylation of ERα and tamoxifen resistance.

DISCUSSION

In this study, we demonstrate for the first time the role of IKKε in ERα activation and tamoxifen sensitivity in breast cancer. Whereas IKKα is a member of IKK family, it only shares 30% amino acid identity to IKKα and IKKβ in their kinase domains. IKKε has been shown to activate NFκB; however, it differs from the IKKα-IKKβ-IKKγ complex. In response to
proinflammatory stimuli, IKKα and IKKβ phosphorylate Ser-32/36 of IκB leading to NFκB activation (28). Whereas, IKKε only phosphorylates IκB Ser-36 to mediate NFκB activation induced by PMA and the activated T-cell receptor (10, 12). In addition, IKKε phosphorylates interferon response factors 3/7 (IRF3 and IRF7), STAT1, and tumor suppressor CYLD to regulate the type I interferon response and cell transformation, respectively (13, 28, 29). We have shown in this report that IKKε phosphorylates ERα-Ser-167 in vitro and in vivo, leading to activation of ERα and up-regulation of cyclin D1. In consideration of frequent overexpression/activation of IKKε in breast and ovarian cancer as well as its ability to transform HMEC-MEK1 cells (15–17), these findings suggest that IKKε exerts its cellular function through regulation of not only canonical NFκB pathway but also other important cascades.

Tamoxifen has been the mainstay of hormonal therapy in the treatment of breast cancer. Despite its long-term benefit, some tumors eventually become resistant to tamoxifen and exhibit an estrogen-independent phenotype even though ERα is maintained in a subset of cases. One of the underlying molecular

![Knockdown of IKKε sensitizes cells to tamoxifen-induced cell death](image)

**FIGURE 6.** Knockdown of IKKε sensitizes cells to tamoxifen-induced cell death. A, knockdown of IKKε. T47D cells were infected and transfected with lentivirus/shRNA-IKKε and siRNA-IKKε, respectively. As controls, the cells were also treated with shRNA-GFP and scramble/mismatched siRNA-IKKε. The methods and nucleotide sequence were described under “Experimental Procedures.” The knockdown of IKKε and its effect on pER-Ser-167 were assessed by immunoblotting with the indicated antibodies. B–D, depletion of IKKε enhances the tamoxifen effect on total cell death, apoptosis, and focus formation. IKKε knockdown and control cells were treated with the indicated concentration of tamoxifen and subsequently assayed for total cell death, apoptosis, and focus formation as described in the legend to Fig. 5. Asterisks indicate p < 0.05. E, IKKε expression levels correlate with pERα-Ser-167 and tamoxifen sensitivity in ERα-positive breast cancers. Representative ERα-positive breast cancer specimens were immunoblotted with indicated antibodies. Tumors expressing high levels of IKKε increase pERα-Ser-167 and are insensitive to tamoxifen indicated by arrows.
mechanisms is the phosphorylation of ERα by protein kinases, which include serine/threonine kinases Erk1/2 (Ser-118, Ref. 22), Akt, pp90Rx1, and S6K1 (Ser-167, Ref. 6, 23, 24), cyclin A/CDK2 (Ser-104/106, Ref. 25), PKA (Ser-236, Ref. 30) and p38 (Thr-311, Ref. 31) as well as tyrosine kinase c-Src (Tyr-537, Ref. 8). These phosphorylation events induce ERα activity and result in tamoxifen resistance. The ERα has an N-terminal domain with a hormone-independent transcriptional activation function (AF-1, amino acids 1–180), a central DNA-binding domain (amino acids 181–263) and a C-terminal ligand-binding domain with a hormone-dependent transcriptional activation function (AF-2; amino acids 302–552, Ref. 32, 33). Because Ser-167 is located in the AF-1 region, activation of ERα by phosphorylation of Ser-167 is ligand-independent. We demonstrated that expression of IKKε sufficient to induce ERE-Luc activity and cyclin D1 expression in ERα-positive cells.

Our data also show that expression of IKKε alone in ERO-negative cells induces cyclin D1 expression and its promoter activity (Fig. 4, B and C), suggesting that IKKε regulates cyclin D1 not only through phosphorylation of ERα but also through other molecules. It has been well documented that the NFκB pathway transcriptionally regulates cyclin D1 (34–36). Thus, IKKε-induced cyclin D1, in addition to ERα, is also mediated through activation of the NFκB pathway.

It has been shown that the PI3K inhibitor LY29002 and mTOR inhibitor rapamycin efficiently inhibit Akt- and S6K1-induced ERα activation and cell proliferation, respectively (18, 24). Therefore, development of small molecule inhibitors of IKKε could have great potential to surmount IKKε-associated tamoxifen resistance. Previous studies have demonstrated that inhibition of IKKε by either shRNA or dominant-negative IKKε-K38A reduces cell growth, survival, and invasion and that ectopic expression of IKKε induces cell survival and proliferation (15, 17). These findings indicate that IKKε could be a critical therapeutic target. Further investigations are required to identify small molecule inhibitor(s) of IKKε for anticancer drug discovery and elucidate IKKε oncogenic activity in transgenic mouse models.

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