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Rapid activation of ERK1/2 and AKT in human breast cancer cells by cadmium

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Abstract

Cadmium (Cd), an endocrine disruptor, can induce a variety of signaling events including the activation of ERK1/2 and AKT. In this study, the involvement of estrogen receptors (ER) in these events was evaluated in three human breast caner cell lines, MCF-7, MDA-MB-231, and SK-BR-3. The Cd-induced signal activation patterns in the three cell lines mimicked those exhibited in response to 17β-estradiol. Specifically, treatment of MCF-7 cells, that express ERα, ERβ and GPR30, to 0.5-10 μM Cd for as little as 2.5 min resulted in transient phosphorylation of ERK1/2. Cd also triggered a gradual increase and sustained activation of AKT during the 60 min treatment period. In SK-BR-3 cells, that express only GPR30, Cd also caused a transient activation of ERK1/2, but not of AKT. In contrast, in MDA-MB-231 cells that express only ERβ, Cd was unable to cause rapid activation of either ERK1/2 or AKT. A transient phosphorylation of ERα was also observed within 2.5 min of Cd exposure in the MCF-7 cells. While the estrogen receptor antagonist, ICI 182,780, did not prevent the effect of Cd on these signals, specific siRNA against hERα significantly reduced Cd-induced activation of ERK1/2 and completely blocked the activation of AKT. It is concluded that Cd, like estradiol, can cause rapid activation of ERK1/2 and AKT and that these signaling events are mediated by possible interaction with membrane ERα and GPR30, but not ERβ.

Keywords

Cadmium; ERK1/2; AKT; Endocrine Disruptors; Estrogen Receptor; GPR30

Introduction

Cadmium (Cd) is a heavy metal that is widely distributed throughout the environment as a result of pollution from a variety of sources. The environmental exposure to Cd occurs through contaminated food and cigarette smoke. For the general population, tobacco smoking is an important source of Cd exposure (WHO/IPCS, 1992). Cd is classified as a category I carcinogen by the International Agency for Research on Cancer (IARC, 1993). However, in comparison with the other carcinogenic metals, Cd is not a strong cancer initiator and instead, it is a weak mutagen (Rossman et al., 1992). Cd-induced stimulation of cellular signaling at various stages of the mitogenic cascade, proto-oncogene expression,

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DNA synthesis, and cell proliferation in various mammalian cell systems is thought to be the mechanisms for the carcinogenic action of this metal.

During the last decade, a growing body of evidence has accumulated suggests that in certain cell types micromolar concentration of Cd is able to initiate a series of rapid signaling events including: generation of second massagers (IP3, cAMP) within 15-30 sec (Smith et al.,1989; Yamagami et al., 1998); increase in intracellular calcium within 3 min (Smith et al.,1989; Yamagami et al., 1998); activation of certain kinases (Hung et al., 1998; Galan et al., 2000; Misra et al., 2002; 2003); and induction of early response genes c-fos and c-myc within 30 min (Beyersmann and Hechtenberg, 1997; Misra et al., 2002; 2003). Since these signals were generated soon after Cd exposure, it argued against a physiological interaction between Cd and intracellular proteins. It has been postulated that Cd might bind to and activate a putative cell membrane receptor and trigger the downstream signaling events. However, only a limited number of studies (Misra et al., 2002; 2003) have explored this possibility, and there is no definitive study that elucidates the nature of the receptor that might possibly mediate this effect.

Estrogen can modulate cellular functions through non-genomic actions which are initiated from the cell membrane. There are two proposed models for the rapid response to estrogen action. The first model proposes that the membrane-bound estrogen receptor alpha (mERα) is associated with the cell membrane proteins, including G protein and receptor tyrosine kinase, and mediates the rapid signaling induced by estrogen (Razandi et al., 2003a, b; 2004; Pedram et al., 2006). The second model proposes that another membrane G protein-coupled protein, GPR30 is involved in this process (Filardo et al., 2000; 2002; Norman et al., 2004; Hewitt et al., 2005; Revankar et al., 2005; Thomas et al., 2005). Although the nature of mERα is still debated (Song and Santen, 2006; Warner and Gustafsson 2006), there is evidence that in certain cell types, a small fraction of ERα, that is associated with the cell membrane and originates from the same transcript of classical ERα, is required for rapid effects of estrogen (Razandi et al., 2003a, b; 2004; Song et al., 2004; Pedram et al., 2006).

At relatively low doses (5-10 μg/kg, ip), Cd is able to increase uterine wet weight, promote growth and development of mammary gland, and induce hormone-regulated genes in ovariectomized rats (Johnson et al., 2003). As a xenoestrogen, Cd has the ability to bind to ERα with high affinity (Kd = 4.5×10^{-10}), and activate and alter estrogen target gene transcription and expression (Garcia et al., 1994; Stoica et al., 2000). If the non-genomic effects of E2 are mediated by membrane ERα (mERα), it is conceivable that Cd may also trigger a rapid signaling cascade by the same mechanism.

Mitogen activated protein kinases (MAPKs) are serine/threonine protein kinases that play important roles in the signaling cascades regulating cellular process such as cell proliferation, differentiation and apoptosis. Three major subfamilies have been described: extracellular-regulated kinases (ERK), c-JUN N-terminal kinase (JNK), and p38 kinase. Depending on the cellular context and stimulators, there is a significant cross talk between transduction modules as they can respond to a common upstream activator and phosphorylate common down stream target. In general, the ERK pathway is preferentially activated in response to growth and differentiation factors, while JNK and p38 kinase are more sensitive to stress stimuli including heat shock, inflammatory cytokines, ultraviolet and gamma irradiation, and hyperosmolarity (Roux and Blenis, 2004). The cells show different temporal and dose-response patterns to Cd treatment in terms of activation of MAPKs. Among the three major subfamilies of MAPKs, ERK1/2 is usually activated by Cd much earlier than JNK and p38 kinase (Alam et al., 2000; Chuang et al., 2000; Chuang and Yang, 2001; Huang et al., 2001; Iryo et al. 2000). Additionally, rapid activation of ERK1/2 is

widely used as an endpoint to reflect E2-induced non-genomic effects in a variety of cell types (Filardo et al., 2000; 2002; Razandi et al., 2003a, b; 2004; Pedram et al., 2006).

The activation of either ERK1/2 or AKT, or both pro-survival kinases, could prevent cells from undergoing apoptosis induced by certain chemical or physical agents. The serine/ threonine protein kinase PI3K/AKT is also activated by various growth and survival factors. AKT promotes cell survival through two distinct pathways: inhibition of apoptosis by phosphorylation of the Bad component of the Bad/Bcl-XL complex, causing dissociation of the Bad/Bcl-XL complex and allowing cell survival; and activation of IKK that ultimately leads to NF-kB activation and cell survival (Hennessy et al., 2005). E2 can induce the activation of AKT in endothelial cells from wild type mice within 15 min, but not in cells from ERα/ERβ combined-deleted mice (Pedram et al., 2006). Misra et al. (2003) reported that in 1LN prostate cells AKT was activated by 1 μM Cd within 20 min.

The present study was designed to investigate rapid signaling in response to Cd in three human breast cancer cell lines with different expression profiles for ERα, ERβ and GPCR30.

Materials and methods

Chemicals and biochemicals

17β-estradiol (E2) and phenol red-free DMEM/Ham's F12 were obtained from Sigma-Aldrich (St. Louis, MO). Fetal bovine serum (FBS) was purchased from HyClone (Logan, UT). The synthetic estrogen antagonist ICI 182,780 was purchased from Tocris Chemicals (Ellisville, MO). Antibodies against phospho-ERK1/2 (Thr202/Tyr204), phospho-AKT (Ser473), phospho-ERα (Ser118), ERK2, and AKT were purchased from Cell Signaling Technology (Danvers, MA). The Anti-ERα (HC-20) antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, California). Blocking buffer was obtained from Li-Cor (Lincon, NE). For siRNA studies, a smart pool of double-stranded siRNA against human ERα (hERα; M-003401-02) and non-specific siRNA (D-001206-01-05) were obtained from Dharmacon Tech (Lafayette, CO). The siRNA transfection reagent siPORT lipid was purchased from Ambion (Austin, TX). All the other chemical reagents were either from Sigma-Aldrich (St. Louis, MO) or Fisher Scientific (Suwanee, GA).

Cell culture

The three breast cancer cell lines, MCF-7 (ERα-, ERβ-, and GPR30-positive), MDA-MB-231(ERβ-positive), and SK-BR-3 (GPR30 positive), were purchased from American Type Culture Collection (Mannassas, VA). The cells were grown and maintained in phenol red-free DMEM/Ham's F12 medium (1:1) containing 1.2 g/L sodium bicarbonate, 10% FBS and 50 mg/L gentamicin (Filardo et al., 2000; 2002). Stock cultures were maintained in the complete medium in a humidified atmosphere of 95% air-5% CO_2 at 37°C and employed for experiments within 15 serial passages. The cells were sub-cultivated every 6-7 days using a mixture of 0.25% trypsin and 0.03% EDTA.

Cell exposure and preparation of cell extract

The cells (1×10^5/cm^2) were seeded in T-75 flasks or 6-well cell culture plates in phenol red-free DMEM/F-12 medium containing 10% FBS. The next day, the medium was replaced with the phenol red-free, serum-free medium and the cells were maintained for 48 h. Treatment of quiescent cells (60%-80% confluences) with Cd was carried out at 37°C in phenol red-free, serum-free, medium as described in the figure legends. Since 10 μM Cd has been commonly used by other researchers for studying the effects of Cd on MAPKs (Chuang et al., 2000; Chuang and Yang 2001; Huang et al., 2001; Iryo et al. 2000), the same

concentration was used in the present study for comparison with the earlier reports. As determined by the MTT and trypan blue exclusion assays, treatment of cells with 10 μM Cd for 60 min did not cause cytotoxicity (data not show). At the end of Cd treatment, the cells were washed twice with ice-cold phosphate-buffered saline (PBS) and lysed with ice-cold RIPA buffer (150 mM sodium chloride, 100mM Tris, pH 7.5, 1% deoxycholatic sodium, 0.1% sodium dodecyl sulfate (SDS), 1% Triton X-100, 100 mM sodium pyrophosphate, 3.5 mM NaVO$_4$, 2 mM PMSF, 50 mM NaF, 2 mM EDTA, plus protease inhibitor cocktail at a ratio of 1000:1). The crude cell lysates were centrifuged at 13,000g for 15 min and the supernatants were stored at -80°C until analyzed. The protein concentration was determined using micro BCA reagent kit from Pierce Biotechnology (Rockford, IL) according to the manufacturer's instructions.

Western blot analysis

Prior to electrophoresis, the cell extracts were heated at 95-100°C in Laemmli buffer containing 5% β-mecaptoethanol for 5 min. The amount of protein in the extract used for electrophoresis was kept constant in the same experiment but varied between the experiments (15-30 μg). After electrophoresis, the proteins were electrotransferred to nitrocellulose membrane at 20 V for 20 min by using Trans-Blot SD Semi-dry Transfer Cell (Bio-Rad, Hercules, CA). The membranes were then blocked with the Li-Cor blocking buffer for 1 h at room temperature and incubated with primary antibodies diluted in PBS-0.1% Tween-20 containing 2.5% BSA (PBST-BSA) overnight at 4°C or for 1 h at room temperature. The nitrocellulose membranes were washed and incubated with secondary antibody labeled with Alexa Fluor 680 (1:15,000 diluted in blocking buffer containing 0.1% Tween-20) for 1 h followed by extensive washing with PBS-0.1% Tween-20. The washed membranes were scanned with the Odyssey Infrared Imager (Li-Cor, Lincon, NE) and the optical density of the bands was quantified by using the application software version 1.2.15.

Transfection with the small interfering RNA (siRNA)

The siRNA transfection was conducted according to the instructions provided by Dharmacon Tech (Lafayette, CO). Briefly, the cells were transfected with either non-specific siRNA (control) or siRNA against human ERα (hERα), and then recovered overnight. The cells were serum-deprived for 24 h prior to treatment with Cd.

Data analysis

Each experiment was repeated three times and the optical density data were statistically analyzed for significant differences by one-way ANOVA, followed by the Tukey-Kramer test, at p<0.05.

Results

Activation of ERK1/2 by Cd

The presence of phosphorylated threonine and tyrosine residues in MAPK correlates with the activities of kinases. Thus, to assess activated ERK1/2 levels, the cell extracts were processed using Western blot analyses using antibodies against phosphorylated peptides encompassing ERK1/2 residues. First, the time-course of Cd-induced ERK1/2 activation was compared between the MCF-7, MDA-MB-231, and SK-BR-3 cells. E2 was used as a positive control and response pattern comparisons were made between E2 (10 nM) and Cd (10 μM). The effect of medium change on the ERK1/2 levels was examined in untreated MCF-7 cells and no significant changes were observed (data not shown). As depicted in Fig. 1A, B, G, and H, the temporal response pattern of ERK1/2 activation induced by Cd in

MCF-7 cells was similar to that induced by E2. Maximal ERK1/2 activation by both E2 and Cd was observed at 2.5 min followed by a gradual decrease over the 60 min period. In contrast to the MCF-7 cells, neither Cd nor E2 activated ERK1/2 in the MDA-MB-231 cells that express only ERβ (Fig. 1C, D, G, and H). However, in the SK-BR-3 cells, that express only GPR30, both Cd and E2 caused transient activation of ERK1/2 (Fig. 1E, F, G, and H), similar to that observed in the MCF-7 cells. These results indicated that mERα and GPR30, but not ERβ were important in Cd-induced rapid signaling of ERK1/2.

The concentration-response data of MCF-7 cells to 0.5-10 μM Cd are shown in Fig. 2A and B. The cells exposed to the lowest concentration of Cd (0.5 μM) for 2.5 min showed a significant increase (2.2-fold) in ERK1 activation. The maximum activation (6.3-fold) was seen at the 5-10 μM Cd.

Activation of AKT by Cd

Since PI3K/AKT is activated through phosphorylation of threonine by an upstream kinase, the activation of AKT can be detected by Western blot analysis using a phospho-specific antibody that recognizes the peptide containing threonine residues. As with ERK1/2, medium change had no significant effect on the AKT levels in the untreated MCF-7 cells (data not shown). As shown in Fig. 3A and G, Cd significantly increased (3.3-fold) the phosphorylated AKT in MCF-7 cells within 2.5 min and continued to do so (5.4-fold) for the duration of the experiment. The E2- induced AKT activation showed a similar pattern (Fig. 3B and H). In the MDA-MB-231 cells, Cd but not E2 induced a moderate activation of AKT only at 60 min (Fig 3C, D, G and H). In comparison, AKT phosphorylation in the SK-BR-3 cells was not affected by either Cd or E2 at any time, similar to the response of these cells to ERK1/2 phosphorylation. Thus, these data also pointed to the involvement of mERα in AKT signaling by Cd.

Phosphorylation of ERα by Cd

The results obtained for both ERK1/2 and AKT phosphorylation in the MCF-7 cells suggested that mERα might play a role in Cd-induced rapid signaling. Thus, to further delineate its involvement in Cd-induced signaling, the phosphorylation of ERα was investigated in quiescent MCF-7 cells after treatment with Cd and E2. As shown in Fig. 4A and C, Cd transiently elevated (3.9-fold) the level of phosphorylated receptor at 2.5 min. In comparison, E2 caused a gradual and more sustained ERα phosphorylation (Fig. 4B).

Effect of an estrogen antagonist on Cd-induced ERK1/2 and AKT activation

Since E2-induced rapid activation of ERK1/2 in MCF-7 cells is through mERα, ICI 182,780, an estrogen antagonist, is able to antagonize both E2-induced genomic and non-genomic rapid signaling effects. In the present study, the possible role of mERα in Cd-induced rapid signaling was examined using the same antagonist. The quiescent MCF-7 cells were pretreated with 10 μM ICI 182,780 for 15 min, and then treated with 10 μM Cd or 10 nM E2 for 2.5 min in the presence of the antagonist. Since ICI 182,780 stock solution (10 mM) was prepared in ethanol, the control cells were incubated with medium containing 0.1% ethanol. As depicted in Fig. 5A-D, whereas the antagonist completed blocked the E2-induced phosphorylation of both kinases. It had no significant effect on the Cd-induced phosphorylation of either kinase. Lack of effect of Cd-induced activation of ERK1/2 and AKT by the receptor antagonist was contrary to all the other results obtained thus far and suggested an mERα-independent mechanism.

Effect of siRNA against hERα on Cd-induced ERK1/2 activation

To further evaluate the role of mERα in Cd-induced rapid activation of ERK1/2 and AKT, a second approach was utilized in which specific siRNA was used to knockdown the expression of the receptor. Transfection with hERα siRNA markedly, but not completely, suppressed the expression ERα in quiescent MCF-7 cells (Fig. 6A and B). Whereas this level of suppression of ERα expression was sufficient to completely block the E2-induced rapid activation of both ERK1/2 and AKT (Fig. 6A-D), it only partially blocked (60%) the Cd-induced activation of ERK1/2 (Fig. 6A and C). However, it completely blocked the activation of AKT (Fig. 6B and D). These results suggest that, in addition to mERα, other receptors might also be involved in interacting with Cd.

Discussion

Cd has been described as an endocrine disrupting chemical by a number of investigators (Takiguchi and Yoshihara, 2006; Henson and Chedrese, 2004). As a xenoestrogen, Cd can alter the expression of certain estrogen target genes and affect breast tissue development in ovariectomized rats (Johnson et al., 2003). Other metals, such as arsenic and uranium have also been evaluated for the reproductive tissue effects, but Cd is the only one with demonstrated effects on mammary proliferation (Johnson et al., 2003; Fenton, 2006). Although there is no definitive epidemiological evidence showing a positive relationship between Cd exposure and breast cancer incidence in the general population (Nagata et al., 2005; McElroy et al., 2006), as a xenoestrogen, Cd may play a role in cancer promotion and progression after the cancer is initiated (Brody and Rudel, 2003; Yager and Davidson, 2006). The mechanism by which Cd disrupts endocrine function remains poorly understood.

The results obtained in the present study demonstrated that in MCF-7 cells, a relatively benign breast cancer cell line, Cd was able to rapidly activate ERK1/2, AKT and ERα at a micromolar concentration within 2.5 min. Under the same exposure condition, SK-BR-3 cells, a moderately malignant breast cancer cell line, responded a bit more slowly (7.5 min) in terms of ERK1/2 activation. In comparison, a highly invasive breast cancer cell line, MDA-MB-231, did not show a rapid response to Cd treatment at all. From the dramatic response differences between these three cell lines, it might be speculated that sensitivity to Cd might be associated with the relative invasiveness of the breast cancer cells.

Since activation of both ERK1/2 and AKT plays a role in regulating various cellular processes such as cell growth, cell differentiation, and apoptosis induced by certain chemical or physical agents, more studies need to be performed to further elucidate how Cd-induced rapid signaling affects the normal function of breast tissue and it's susceptibility to certain stresses. In the present study, Cd also induced rapid phosphorylation of ERα, which might make the cells more sensitive to relatively low levels of E2. Although the downstream effects were not investigated in the present study, the results of ERK1/2, AKT and ERα phosphorylation suggest that even occasional exposure to Cd might cause transient effects on the normal physiology of the breast tissue and might disrupt the normal homeostasis, especially if the exposure occurs in early critical period of breast development (Fenton 2006). The potential short-term exposure to low concentration of Cd is of environmental health importance and may have biological significance, especially when the subjects are co-exposed to other chemicals or physical stress agents (Brody and Rudel, 2003).

The results presented in this study also showed that Cd, like E2, caused rapid phosphorylation of ERK1/2 in MCF-7 cells only 2.5 min after treatment. This suggests that Cd and E2 possibly share a similar downstream signal transduction pathway and regulatory mechanism. The rapid inactivation of ERK1/2 implies the existence of a tightly controlled regulatory mechanism. In comparison with the present study, Silva et al. (2006) reported

that 0.1 μM Cd did not induce significant activation of ERK1/2 in MCF-7 BUS cells during the 5-20 min treatment period. Although the differences in biological characteristics and sensitivity to E2 and endocrine disruptors among different MCF-7 cell stocks may partially contribute to the differences in Cd-induced rapid activation of ERK1/2 (Villalobos et al., 1995), it is also possible that 0.1 μM Cd is too low a concentration to detect any significant effects. In this regard, the present study provided a more comprehensive concentration-response data about Cd-induced rapid signaling in the MCF-7 cells between 0.5 and 10 μM concentration.

Alam et al. (2000) reported increased phosphorylation of ERK1/2 in the MCF-7 cells in response to Cd starting at 15 min and peaking at 1 h. They used MCF-7 N variant cells which could partly account for their results and those reported in the present study (Burow, et al., 1998). Secondly, these investigators used DMEM containing phenol red, and performed 24 h serum starvation before exposure. The present study utilized phenol red-free DMEM/F-12 medium and serum starved the cells for 48 h. Because phenol-red is a weak estrogen mimic, it can interfere with the effects of estrogen. Therefore, phenol-red should be excluded from the medium when studying the effects of estrogen or endocrine disruptors. Additionally, since ERK1/2 can be activated very rapidly, early time points need to be considered in designing such studies.

Guerra et al. (2004) observed that short treatment of septal murine cells (SN56) with E2 caused a biphasic activation of ERK1/2, with peak induction at 4-8 min in the early phase and 8 h in the second phase. Similarly, Marino et al. (2003) reported that treatment of HepG2 cells with E2 also caused a biphasic phosphorylation of AKT with peaks at 3 and 30 min, respectively. Furthermore, that the first wave of activation was ER-independent, whereas the second was ER-dependent.

Without serum starvation, 20 μM Cd induced significant phosphorylation of ERK1/2 in MCF-7 cells only after 18 h of exposure (Matsuoka and Igisu, 2001). Brama et al. (2007) also studied Cd-induced activation of ERK1/2 and AKT in the MCF-7 cells and reported maximum activation between 3 and 6 h. They concluded that this activation occurred through an ERα-dependent mechanism, because it could be fully blocked by ICI 182,780. In comparison, in the present study, Cd caused rapid activation of ERK1/2 at 2.5 min in the MCF-7 cells and while it was not blocked by ICI 182,780, it was partially blocked in cells transfected with by hERα. Thus, Cd like E2 induces a biphasic activation of ERK1/2; however, both phases might involve ERα.

Besides the breast cancer cells, the activation of ERK1/2 by Cd has been reported in several other cell types, including CCRF-CEM cells, non-small-cell lung carcinoma cell line CL3, and mouse epidermal JB6 C141 cells (Iryo et al., 2000; Chuang et al., 2000; Chuang and Yang, 2001; Huang et al., 2001). The phosphorylation of ERK1/2 in the CCRF-CEM cells treated with 10 μM Cd occurred after 30 min exposure (Iryo et al., 2000). In non-small-cell lung carcinoma cell line CL3, high concentration of Cd (>80 μM) induced phosphorylation of ERK1/2 after 6 h of treatment (Chuang et al., 2000). Similarly, in mouse epidermal cells, Huang et al. (2001) reported that 1 μM Cd induced a sustained activation of ERK1/2 between 20 min and 9 h. Wang and Templeton (1998) reported that ERK1/2 activation in rat mesangial cells was involved in Cd-induced c-fos mRNA transcription and that inhibition of ERK1/2 partially attenuated Cd-induced c-fos transcription. Thus different cell lines show variations in response to Cd-induced activation of ERK1/2 in terms of time-course and concentration-dependence. It is likely that multiple mechanisms are involved in mediating this effect and that certain biological responses are cell type specific.

An important finding in this study was the MDA-MB-231 cells that express only ERβ did not respond to Cd, ruling out the involvement of this receptor in Cd signaling. GPR30 has been shown to be involved in rapid E2 signaling events (Filardo et al., 2000; 2002). Since these cells responded to Cd, GPR30 also appears to interact with Cd.

In conclusion, the results presented here provide evidence that micromolar concentration of Cd rapidly activate ERK1/2 and AKT in the MCF-7 and SK-BR-3 cells. This process seems to involve mERα and GPR30. It is plausible that Cd could also bind to other membrane receptors, upstream kinases or G-protein coupled proteins, which are associated with specialized cell membrane structural complexes, such as caveolae, causing rapid activation of downstream kinase cascade (Wong et al. 2002; Chini and Parenti, 2004). Further studies are needed to elucidate the mechanisms of Cd-induced rapid signaling and to better understand how Cd interferes with the cell signaling network, affecting normal physiological function and homeostasis.

Acknowledgments

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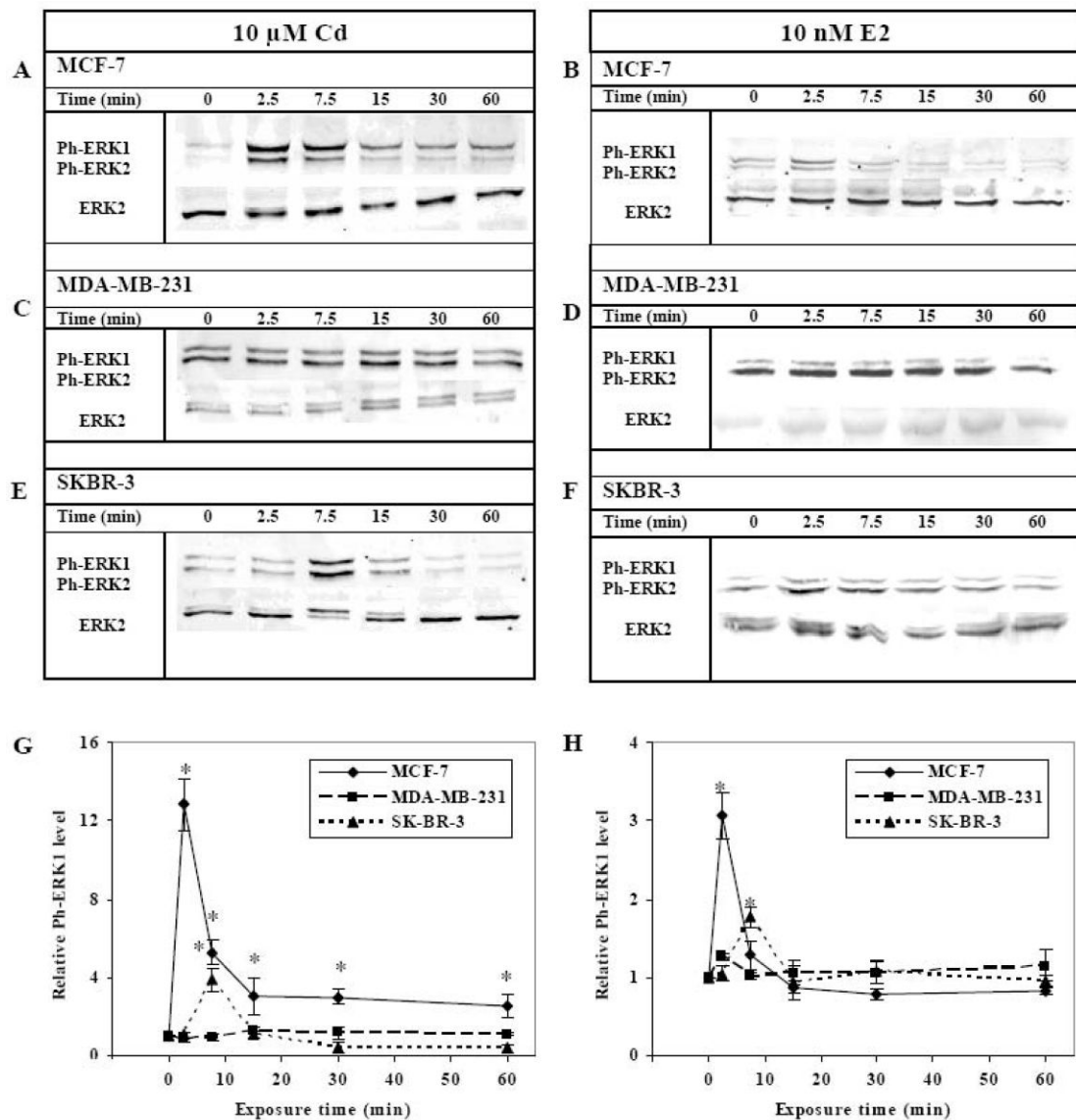
Fig. 1.
Time-course of ERK1/2 activation by Cd and E2 in three different breast cancer cell lines. (A, B) MCF-7 cells, (C, D) MDA-MB-231 cells, and (E, F) SK-BR-3 cells. The quiescent cells were treated with 10 µM Cd or 10 nM E2 for up to 60 min. Comparison of temporal response patterns of Cd- and E2-induced ERK1/2 activation in the three cell lines is shown in G and H. Data are presented as mean±SD from three independent experiments. *Significantly higher than the basal level for the respective cells ($p < 0.05$).
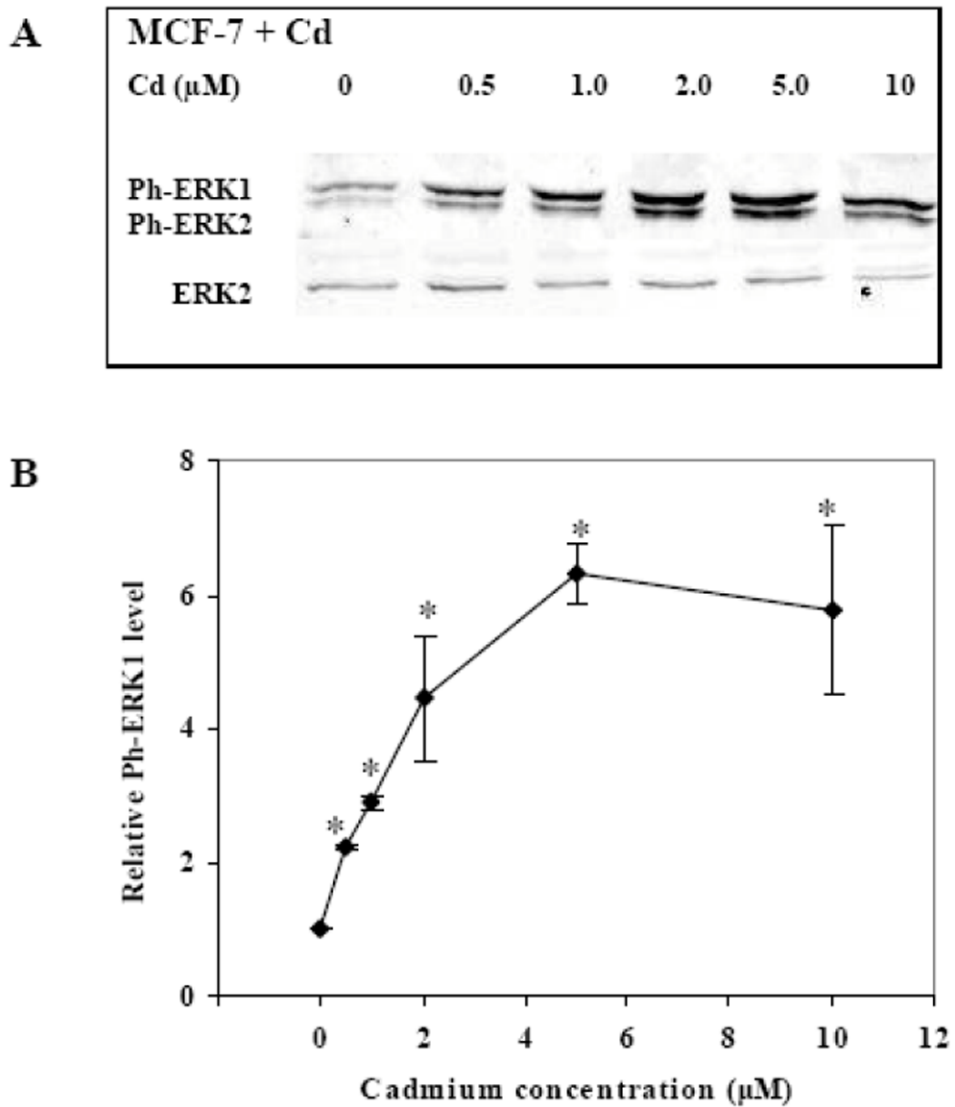
Fig. 2.
Concentration-response pattern of ERK1/2 activation by Cd in MCF-7 cells. Quiescent MCF-7 cells were treated with up to 10 µM Cd for 2.5 min. Data are presented as mean±SD from three independent experiments. *Significantly higher than the basal level ($p < 0.05$).
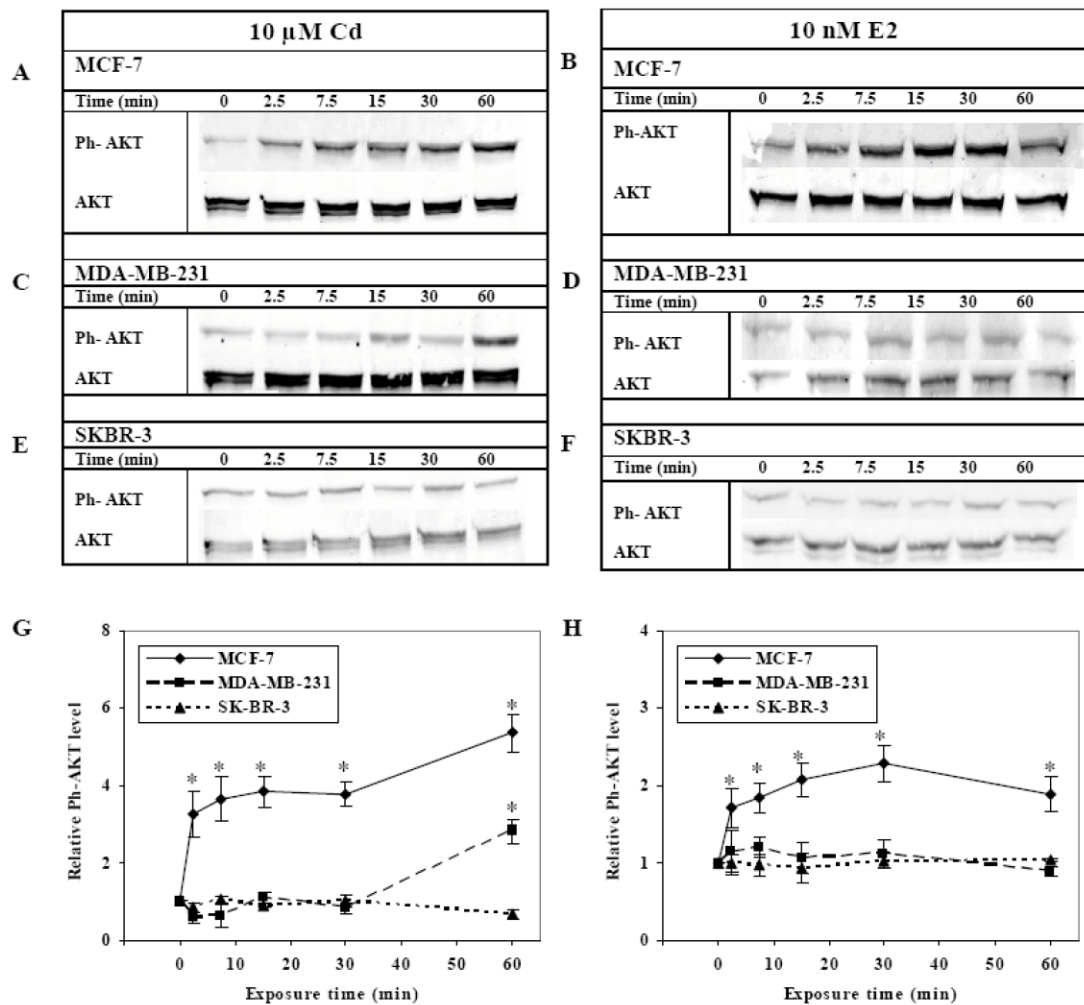
Fig. 3.
Time-course of AKT activation by Cd and E2 in three different breast cancer cell lines. (A, B) MCF-7 cells, (C, D) MDA-MB-231 cells, and (E, F) SK-BR-3 cells. The quiescent cells were treated with 10 μM Cd or 10 nM E2 for up to 60 min. Comparison of temporal response pattern of Cd- and E2-induced AKT activation in the three cell lines is shown in G and H. Data are presented as mean±SD from three independent experiments. *Significantly higher than the basal level for the respective cells ($p < 0.05$).
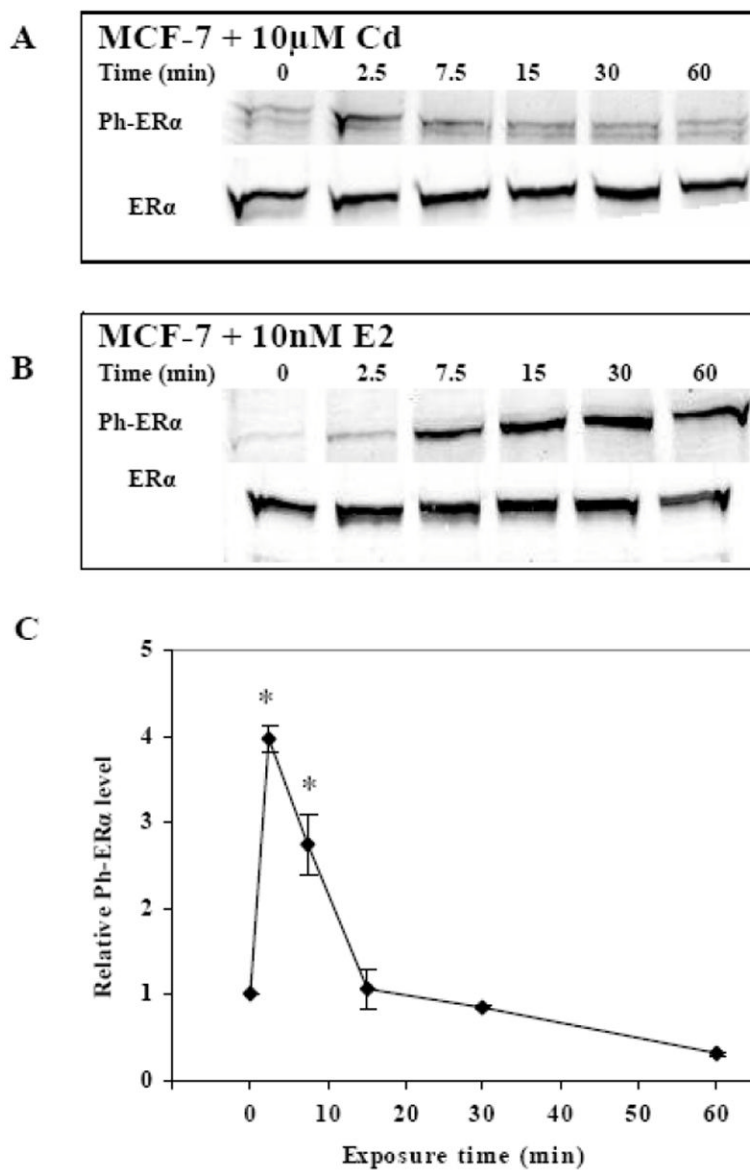
Fig. 4.
Time-course of ERα phosphorylation by Cd and E2 in MCF-7 cells. Phosphorylation of ERα in quiescent cells by: (A, C) 10 µM Cd, and (B) 10 nM E2. Temporal response pattern of Cd is shown in C. Data are presented as mean±SD from three independent experiments. *Significantly higher than the basal level ($p < 0.05$).
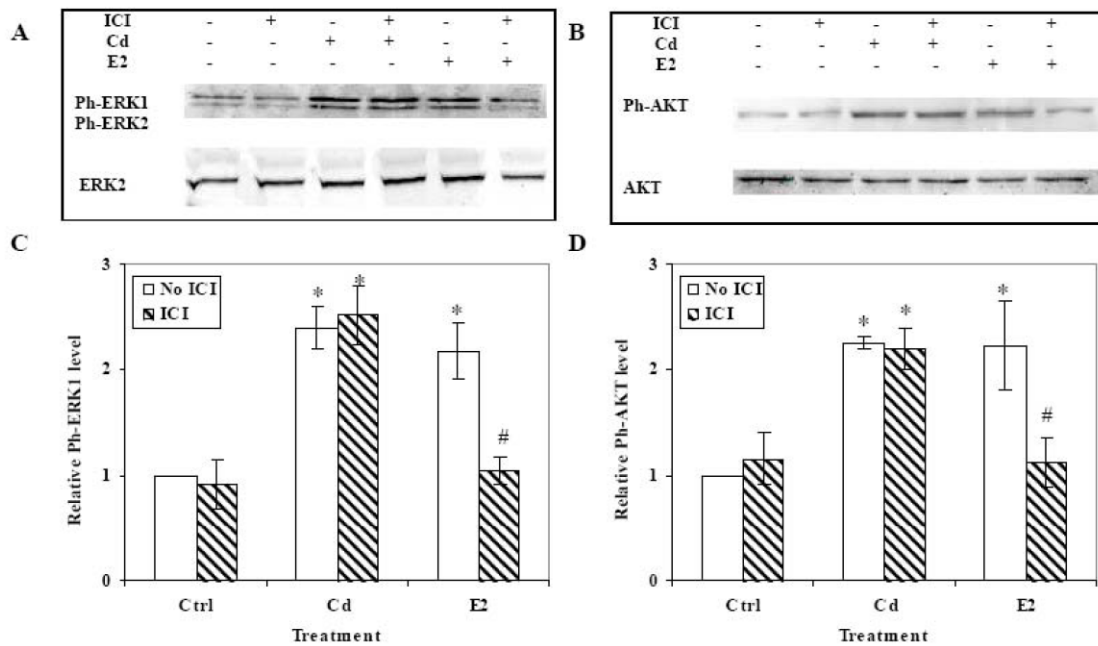
Fig. 5.
Effect of ER antagonist on Cd- and E2-induced activation of ERK1/2 and AKT in MCF-7 cells. The quiescent cells were pretreated with ICI 182,780 (10 μM) or the vehicle (0.1% ethanol) for 15 min and then treated with either 10 μM Cd or 10 nM E2 in the presence of the antagonist for 2.5 min. (A, C) Phosphorylation of ERK1/2, and (B, D) phosphorylation of AKT. Data are presented as mean±SD from three independent experiments. *Significantly higher than the control cells ($p < 0.05$). #Significantly lower than the E2 alone level ($p < 0.05$).
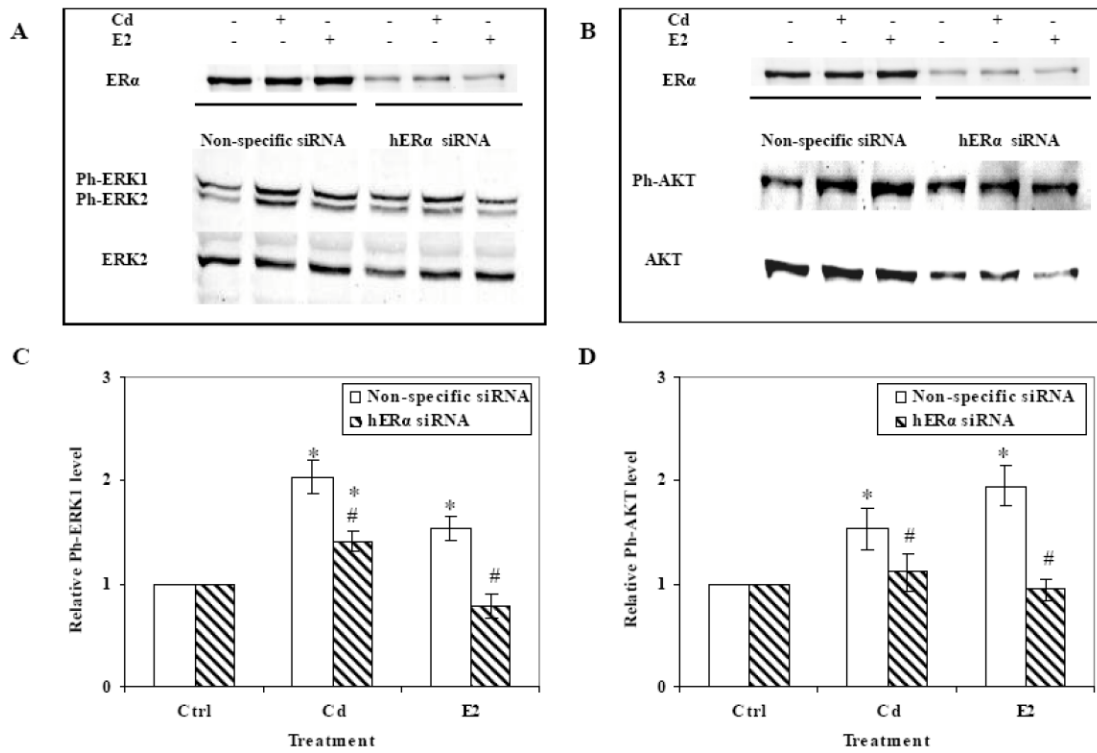
Fig. 6.
Effect of suppression of ERα expression on Cd- and E2-induced activation of ERK1/2 in MCF-7 cells. Forty eight hours after transfection with the non-specific or specific hERα siRNA, the quiescent cells were treated with either 10 µM Cd or 10 nM E2 for 2.5 min. (A, C) Phosphorylation of ERK1/2, and (B, D) phosphorylation of AKT. Data are presented as mean±SD from three independent experiments. *Significantly higher than the respective control cells ($p < 0.05$). [#] Significantly lower than the respective Cd- or E2-treated cells transfected with non-specific siRNA ($p < 0.05$).