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Effects of cadmium on estrogen receptor mediated signaling and estrogen induced DNA synthesis in T47D human breast cancer cells

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Abstract

Cadmium (Cd) has been shown to bind to the human estrogen receptor (ER), yet studies on Cd's estrogenic effects have yielded inconsistent results. In this study, we investigated the effects of Cd on DNA synthesis and its simultaneous effects on both genomic (mediated by nuclear ER (nER)) and non-genomic (mediated by membrane-bound ER (mER)) signaling in human breast cancer derived T47D cells. No effects on DNA synthesis were observed for non-cytotoxic concentrations of CdCl₂ (0.1–1000 nM), and Cd did not increase progesterone receptor (PgR) or pS2 mRNA levels. However, Cd stimulated phosphorylation of ERK1/2 MAPK, detectable following 10 min and 18 h of treatment. The sustained Cd-induced ERK1/2 phosphorylation was inhibited by the ER antagonist ICI 182,780, suggesting the involvement of ER. In addition, Cd enhanced DNA synthesis and pS2 mRNA levels in estrogen (10 pM estradiol) treated T47D cells. The MEK1/2 specific inhibitor U0126 blocked DNA synthesis stimulated by estradiol (E2) and the E2–Cd mixtures. These findings indicate that the ERK1/2 signaling is critical in E2-related DNA synthesis. The sustained ERK1/2 phosphorylation may contribute to the Cd-induced enhancement of DNA synthesis and pS2 mRNA in mixture with low-concentration E2.

Keywords

Cadmium; Estrogen receptor signaling; DNA synthesis; T47D cells

1. Introduction

Estrogen receptor (ER) signaling is involved in many human diseases such as breast cancer (Yager and Davidson, 2006), lung cancer (Stabile and Siegfried, 2004) and cardiovascular diseases (Huss and Kelly, 2004; Meyer et al., 2006). In breast cancer cells, ERs located in the nucleus (nER) transcriptionally regulate the expression of estrogen responsive genes, while

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Conflict of interest

The authors declare that there are no conflicts of interest.

the cell membrane-bound ERs (mER) activate various cytosolic kinase signaling pathways (Acconcia and Kumar, 2006; Levin and Pietras, 2007). Many environmental estrogens can bind with the ER and have been shown to mimic estrogenic effects such as promoting the growth of E2-dependent breast cancer cells (Pillon et al., 2005).

Cadmium (Cd) is a known human carcinogen and environmental exposure is wide-spread (International Agency for Research on Cancer, 1993). Potential mechanisms of Cd carcinogenesis include DNA damage, altered DNA repair, enhanced proliferation and/or depressed apoptosis (Waalkes, 2003). Interestingly, studies have shown that Cd is a potential endocrine disruptor. Cd has been reported to stimulate human breast cancer derived MCF-7 cell growth and also increased mRNA levels of estrogen responsive genes such as the progesterone receptor (PgR) and pS2 (Garcia-Morales et al., 1994). Concerns regarding the potential for Cd as a xenoestrogen stemmed from further evidence provided by the same group that Cd not only bound and activated the ER (Stoica et al., 2000a), but also had in vivo estrogen-like effects in rat uterus and mammary gland (Johnson et al., 2003). The estrogenicity of Cd was further examined by different groups using in vitro assays, but the results were inconsistent. While the estrogenic activity of Cd was confirmed by some groups (Brama et al., 2007; Choe et al., 2003), others reported lack of estrogenicity and an inhibitory effect of Cd on E2-induced reporter activity and cell proliferation (Guevel et al., 2000; Silva et al., 2006). Most available in vitro Cd studies have only investigated its effects at relatively high concentrations (1 μ M and higher). However, according to measurements conducted by NHANES in over 8000 human samples, Cd blood concentration varies from <0.89 (detection limit) to 65 nM (NHANES, 2006), which is much lower than test concentrations used in the studies mentioned above.

In this study, we evaluated the effect on DNA synthesis of low concentrations of Cd in human breast cancer derived T47D cells, whose growth is E2-dependent. We also investigated the effects of Cd on the nER and mER-mediated signaling, as well as its effects on DNA synthesis in mixture with E2 at physiological concentrations.

2. Materials and methods

2.1. Chemicals

17 β -estradiol (E2) and CdCl₂ were purchased from Sigma–Aldrich (St. Louis, MO). The ER antagonist ICI 182,780 was purchased from Tocris (Ellisville, MO). Bromodeoxyuridine (BrdU) was purchased from BD Biosciences (San Diego, CA) and the selective MEK1/2 inhibitor U0126 was obtained from Cell Signaling Technology (Beverly, MA).

2.2. Cell culture and treatment

ER and PgR positive T47D human breast cancer cells were purchased from American Tissue Culture Collection (ATCC, Rockville, MD) and maintained at 37 °C with 5% CO₂ in Dulbecco's modified minimum essential medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Cells were split every 3–4 days and only used from the 1st to 15th passage after receipt from ATCC. The cells were counted and seeded at the following densities: 3 $\times$ 10⁵ cells/well in 6-well plates for ER signaling assays and 1 $\times$ 10⁴ cells/glass coverslip for determination of DNA synthesis. Cells were cultured in DMEM supplemented with 2.5% triple-dextran-charcoal (DCC) stripped FBS for 4 days before treatment with Cd (0.1–1000 nM) or E2 (positive control). In treatments with a Cd–E2 mixture, Cd was added 30 min before E2. The kinase inhibitor U0126 or ER antagonist ICI 182,780 was added 30 min before Cd or E2. Cell viability was checked by Trypan blue staining.

2.3. BrdU incorporation

BrdU labeling was measured to indicate DNA synthesis. In brief, cells were seeded on sterilized glass coverslips in 12-well plates, treated with different test agents for 48 h in the presence of 2.5% DCC-stripped serum, and labeled with 10 μ M BrdU for 4 h. The cells were fixed using 70% ethanol at 4 °C and treated with 2.5N HCl at room temperature for 20 min. After washing with PBS, the cells were incubated with 1:20 anti-BrdU antibody (BD Biosciences) and 1:200 Alexa Fluor 488-labeled anti-mouse IgG antibody (Invitrogen, San Diego, CA), each for 30 min at room temperature in the dark. Following washing and air drying, the coverslips were mounted with Prolong Gold Antifade Reagent containing DAPI (Invitrogen) and observed under a fluorescent microscope. Images of four random fields were taken for later analysis using a digital camera linked to the microscope. The percent BrdU-labeled nuclei were determined by counting 150–250 DAPI stained nuclei within the four fields.

2.4. RNA extraction

Total RNA was extracted using the RNeasy Mini kit (Qiagen, Valencia, CA) according to the protocol provided by the manufacturer. The eluted RNA samples were treated with RQ1 RNase-Free DNase (Promega, Madison, WI). Briefly, an appropriate amount of DNase (1 unit/ μ g RNA) was added to the RNA sample and incubated at 37 °C for 30 min, followed by inactivation by heating at 65 °C for 10 min in the presence of EDTA. The OD_{260/280} ratio of each RNA sample was checked. RNA samples with a ratio higher than 1.8 were used to reverse transcribe cDNA.

2.5. Real-time reverse-transcription PCR

RNA was reverse-transcribed using the Superscript III First Strand cDNA Synthesis Kit (Invitrogen) according to the manufacturer's protocol. Briefly, the reaction was performed in a total volume of 20 μ l with 200 ng of total RNA, 50 μ M of oligo (dT)₂₀, 10 mM of each dNTP, 1 μ l RNase inhibitor, and 200 units of Superscript III reverse transcriptase. The mixture was incubated at 55 °C for 60 min, followed by an inactivation at 70 °C for 15 min. The mRNA levels were determined by real-time PCR with an ABI Prism 7000 sequence detection system (Applied Biosystems, Foster City, CA). Each 20 μ l reaction mixture contained 10 μ l of TaqManUniversal PCR Master Mix (Applied Biosystems), 10 ng cDNA and 1 μ l of the TaqMan Gene Expression primers and probes predesigned by Applied Biosystems. The thermal cycling conditions included an initial denaturation step at 95 °C for 10 min, 40 cycles at 95 °C for 15 s, and 60 °C for 1 min. The fluorescence intensity threshold, at which all samples are compared, was set within the linear segment of the amplification curves. Gene expression analysis, as represented by mRNA levels, was performed using the 2- $\Delta\Delta$ CT method by normalizing to β -actin.

2.6. Western blot analysis for MAPK phosphorylation

Western blots to detect and quantify MAPK phosphorylation were done as described (Song et al., 2002) with modifications. Briefly, at harvest the cells were washed with PBS and extracted using radioimmunoprecipitation (RIPA) lysis buffer supplemented with PMSF, sodium orthovanadate solution, protease inhibitor cocktail, and phosphatase inhibitors (Santa Cruz Biotechnology, Santa Cruz, CA). After centrifugation to remove insoluble material, lysate proteins were quantified by Bradford Assay, separated by 12% SDS-PAGE gels and transferred electrophoretically to nitrocellulose membranes. Activated and total ERK1/2 MAPK were detected using anti-Phospho-p44/42 MAPK (Thr202/Tyr204) mouse antibody and anti-p44/42 MAPK rabbit antibody (Cell Signaling Tech. Beverly, MA), respectively. Near-infrared fluorescence-labeled IRDye 800CW goat anti-mouse secondary antibody and IRDye 680 goat anti-rabbit secondary antibody (Li-Cor Biosciences) were used to detect activated MAPK and

total MAPK, respectively. The membranes were scanned using the LI-COR Odyssey Infrared Imaging System and analyzed using Odyssey software.

2.7. Statistical analysis

Except when indicated, the data are shown as the mean and standard deviation from three independent experiments ($n = 3$), with each data point within an independent experiment determined in duplicate or triplicate. Data was subjected to one-way analysis of variance (ANOVA) followed by the *t*-test with Bonferroni correction if the ANOVA *P* value was less than 0.05. Statistical analysis was performed using Prism software version 4.0 (GraphPad Software Inc., San Diego, CA). For calculation of the effective concentrations of E2-induced DNA synthesis, the data were analyzed by a non-linear regression using Prism 4.0 and fit to a sigmoidal dose-response curve.

3. Results

3.1. Effect of E2 and Cd on DNA synthesis

The effect of Cd and E2 on DNA synthesis in T47D cells was investigated following a 48-h treatment (Fig. 1). Trypan blue exclusion was used to ensure >90% cell viability at the end of treatment at the highest Cd concentration used. BrdU labeling index was not significantly increased by Cd at concentrations up to 1 μ M, while it was stimulated over 4 fold by 0.1 nM E2. The stimulatory effect of E2 could be blocked by the antiestrogen ICI 182,780, demonstrating involvement of the ER.

3.2. Effect of E2 and Cd on PgR and pS2 mRNA levels

Increased PgR and pS2 mRNA levels were used as indicators of a response mediated through the nER, acting through the estrogen response element (ERE). A time course study showed that increased PgR mRNA levels (>2 fold) stimulated by 10 nM E2 were detectable after 18 and 42 h of treatment (Fig. 2A). However, as shown in Fig. 2B, PgR mRNA was not increased in cells treated with Cd for 20 h at concentrations up to 1 μ M. Low concentrations of Cd (up to 0.1 nM) did not increase pS2 mRNA either (Fig. 6A).

3.3. Effect of E2 and Cd on ERK1/2 MAPK phosphorylation

The rapid phosphorylation of ERK1/2 MAPK in MCF-7 cells has been reported as an indicator of membrane ER signaling (Santen et al., 2002; Zivadinovic and Watson, 2005). In T47D cells we observed that both E2 (1 nM) and Cd (0.1–1000 nM) increased ERK1/2 phosphorylation at 10 min compared to the control group that was similarly treated with PBS (Fig. 3).

Since a prolonged ERK1/2 activation was previously reported (Brama et al., 2007), we also measured ERK1/2 phosphorylation after 18 h of exposure to E2 and Cd. As shown in Fig. 4A, after 18 h the E2-induced activation, detected after 10 min (Fig. 3), had diminished to the background level, while Cd-induced activation was prominent and was Cd concentration dependent (densitometric analysis shown in Fig. 4C). The strong ERK1/2 phosphorylation could be detected even after 48 h of Cd exposure (data not shown), suggesting that this effect of Cd was persistent. In addition, this ERK1/2 phosphorylation was inhibited by ICI 182,780, again suggesting involvement of the ER (Fig. 4B and C).

3.4. Effect of Cd on DNA synthesis and gene expression in presence of a low concentration of E2

We also measured the effect of Cd on E2-induced DNA synthesis. Cells were treated with 10 pM E2 in mixture with different concentrations of Cd. E2 itself at this concentration produced about 20% of its maximal effect (Fig. 5 insert). Although Cd itself did not stimulate DNA

synthesis in T47D cells, it significantly ($p < 0.01$) enhanced the effect of 10 pM E2 (Fig. 5). Adding the MEK1/2 specific inhibitor U0126 completely blocked the DNA synthesis induced by E2 and the E2–Cd mixture.

In the presence of 10 pM E2, Cd also significantly ($p < 0.05$) enhanced the induction of pS2 mRNA stimulated by 10 pM E2 alone (Fig. 6A). Further enhancement of PgR mRNA was also observed in cells treated with the Cd–E2 mixture; however, the increase was not statistically significant (Fig. 6B).

4. Discussion

Although Cd showed binding activity to the ER from human and rainbow trout (Nesatyy et al., 2006; Stoica et al., 2000a), studies on Cd's estrogenicity have given inconsistent results (Brama et al., 2007; Choe et al., 2003; Garcia-Morales et al., 1994; Guevel et al., 2000; Reddy et al., 1994; Silva et al., 2006). There is also lack of dose response information, especially on its low-dose effects.

Using human T47D, an ER-positive breast cancer derived cell line whose proliferation is estrogen-dependent, we investigated the effects of Cd on DNA synthesis. We did not observe a proliferative effect of Cd at non-toxic concentrations. This result is in accordance with several previous studies, using either MCF-7 cells (Guevel et al., 2000; Silva et al., 2006) or T47D cells (Reddy et al., 1994). There are other studies showing an ER dependent growth stimulatory effect of Cd in MCF-7 cells (Brama et al., 2007; Choe et al., 2003; Garcia-Morales et al., 1994). However, these studies often lack determination of the dose–response relationship, thus making it difficult to compare with our results. Nevertheless, the above-mentioned discrepancies suggest that the selections of concentration, time, and cell line could influence the evaluation of cell response to Cd and other xenoestrogens.

The cellular responses to E2 and xenoestrogens reflect combined signaling through nER and mER. For nER-mediated effects, it was demonstrated that Cd transcriptionally activated an ERE-linked reporter gene in T47D cells with endogenous ER, and that 1 μ M Cd caused induction of reporter activity comparable to 0.1 nM of E2 (Wilson et al., 2004). In untransfected MCF-7 cells, Stoica et al. reported an ER-dependent increase of PgR mRNA induced by 1 μ M Cd (Stoica et al., 2000b). However, we were unable to detect increased PgR levels induced by Cd at the same concentration in untransfected T47D cells. This discrepancy regarding effects on PgR mRNA may be related to the use of different cell lines in which background PgR mRNA levels are different (Schafer et al., 1999). In addition, since the nER-mediated gene regulation can also act through non-ERE regulated sites, such as AP-1, whether binding with Cd affects the interaction between ER and other transcription factors requires further investigation.

E2 and many xenoestrogens activate multiple mER-mediated cell signaling pathways in breast cancer cells (Levin and Pietras, 2007; Li et al., 2006). It was suggested that MAPK, in particular, plays an important role in the crosstalk between non-genomic ER signaling and gene transcriptional regulation critical to cell proliferation (Atanaskova et al., 2002; Martin et al., 2005; Santen et al., 2002). In recent studies, Cd was shown to stimulate a rapid (Liu et al., 2008), or a sustained (Brama et al., 2007) MAPK phosphorylation in MCF-7 cells. Here we confirmed the existence of both rapid and sustained phosphorylation of ERK1/2 MAPK in another breast cancer cell line, T47D. We demonstrated that the ERK1/2 activation detected after 18 h of Cd treatment was strong and concentration dependent, while E2-induced activation of MAPK was not persistent. In other cell lines such as HEK293, HeLa and HepG2, sustained ERK1/2 phosphorylation was detected following Cd treatment (Martin et al., 2006). Our finding is the first showing that ER antagonist ICI 182,780 could inhibit the Cd-induced ERK1/2 activation, suggesting that in T47D cells this effect was at least partially ER-

dependent. The importance of ERK1/2 signaling in T47D cell proliferation was supported by the observation that the selective MEK1/2 (ERK1/2) inhibitor U0126 completely blocked DNA synthesis induced by E2 and the Cd-E2 mixtures. A study in human breast cancer cells has suggested that estrogen-induced cellular proliferation does not result from early protein kinase activation, but is likely to require the delayed activation of ERK (Geffroy et al., 2005). Similarly, we have observed that DNA synthesis was not induced following a pulse treatment with E2 for 10 min, which is only sufficient to activate the rapid ERK1/2 phosphorylation (data not shown). Therefore, it is likely that cells need more than just the rapid ERK1/2 activation to activate DNA synthesis. The enhanced effect of Cd we observed is likely to be related to the dose-dependent, sustained ERK1/2 phosphorylation, which may crosstalk with the genomic ER activation, thus causing the enhancement of pS2 and PgR mRNA in cells treated with Cd-E2 mixture.

Since humans are exposed simultaneously to endogenous estrogens and environmental agents with estrogenic effects, it has been suggested that environmental estrogens may increase the potential estrogenic burden (Darbre, 2006). Therefore, it is also important to investigate the effects of environmental agents in mixture with E2 (Kortenkamp, 2006). In this study, we examined the effect of Cd on DNA synthesis stimulated by E2 at 10 pM, a concentration comparable to human serum levels and which caused about 20% of the maximal effect that E2 produced in our T47D cells. The concentration range of Cd used in the mixture study was 0–100 nM, which was also within the range of human blood levels mentioned previously. Our study is the first to show a Cd-induced enhancement on the effect of a low concentration of E2. However, a similar effect was observed previously in cells co-treated with Cd and dihydrotestosterone (DHT), a steroid compound with weak estrogenic effect. Using a T47D-KBluc ER activation reporter assay, it was reported that CdCl₂ (1–100 µM) enhanced the effect of DHT in a dose-dependent fashion (Pregnenzer et al., The Toxicologist, 2007, poster #133). Whether Cd has a similar effect on other xenoestrogens such as bisphenol-A and PCBs is worth further investigation.

In summary, we used untransfected T47D cells, whose growth is estrogen-dependent, to study the effect of Cd on DNA synthesis and the nER and mER-mediated signaling. We observed that Cd treatment caused a persistent increase in ERK1/2 phosphorylation and enhanced DNA synthesis and pS2 mRNA stimulated by a low concentration of E2. Future studies should investigate the interaction between Cd and environmental and dietary estrogens. Most of these agents have weak estrogenicity, but together may increase the total estrogen burden. Further studies are also needed using cells of other types to explore the breadth of the types of effects observed here. Such information may help to increase our understanding of the environmental etiology of cancer and in the development of strategies for prevention.

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References

- Acconcia F, Kumar R. Signaling regulation of genomic and nongenomic functions of estrogen receptors. *Cancer Lett* 2006;238:1–14. [PubMed: 16084012]
- Atanaskova N, Keshamouni VG, Krueger JS, Schwartz JA, Miller F, Reddy KB. MAP kinase/estrogen receptor cross-talk enhances estrogen-mediated signaling and tumor growth but does not confer tamoxifen resistance. *Oncogene* 2002;21:4000–4008. [PubMed: 12037682]

- Brama M, Gnessi L, Basciani S, Cerulli N, Politi L, Spera G, Mariani S, Cherubini S, d'Abusco AS, Scandurra R, Migliaccio S. Cadmium induces mitogenic signaling in breast cancer cell by an ERalpha-dependent mechanism. *Mol. Cell Endocrinol* 2007;264:102–108. [PubMed: 17125913]
- Choe S-Y, Kim S-J, Kim H-G, Lee JH, Choi Y, Lee H, Kim Y. Evaluation of estrogenicity of major heavy metals. *Sci. Total Environ* 2003;312:15–21. [PubMed: 12873394]
- Darbre PD. Metalloestrogens: an emerging class of inorganic xenoestrogens with potential to add to the oestrogenic burden of the human breast. *J. Appl. Toxicol* 2006;26:191–197. [PubMed: 16489580]
- Garcia-Morales P, Saceda M, Kenney N, Kim N, Salomon DS, Gottardis MM, Solomon HB, Sholler PF, Jordan VC, Martin MB. Effect of cadmium on estrogen receptor levels and estrogen-induced responses in human breast cancer cells. *J. Biol. Chem* 1994;269:16896–16901. [PubMed: 8207012]
- Geffroy N, Guedin A, Dacquet C, Lefebvre P. Cell cycle regulation of breast cancer cells through estrogen-induced activities of ERK and Akt protein kinases. *Mol. Cell. Endocrinol* 2005;237:11–23. [PubMed: 15925090]
- Guevel RL, Petit FG, Goff PL, Metivier R, Valotaire Y, Pakdel F. Inhibition of rainbow trout (*Oncorhynchus mykiss*) estrogen receptor activity by cadmium. *Biol. Reprod* 2000;63:259–266. [PubMed: 10859267]
- Huss JM, Kelly DP. Nuclear receptor signaling and cardiac energetics. *Circ. Res* 2004;95:568–578. [PubMed: 15375023]
- International Agency for Research on Cancer. Beryllium, Cadmium, Mercury and Exposures in the Glass Manufacturing Industry. 1993.
- Johnson MD, Kenney N, Stoica A, Hilakivi-Clarke L, Singh B, Chepko G, Clarke R, Sholler PF, Lirio AA, Foss C, Reiter R, Trock B, Paik S, Martin MB. Cadmium mimics the in vivo effects of estrogen in the uterus and mammary gland. *Nat. Med* 2003;9:1081–1084. [PubMed: 12858169]
- Kortenkamp A. Breast cancer, oestrogens and environmental pollutants: a re-evaluation from a mixture perspective. *Int. J. Androl* 2006;29:193–198. [PubMed: 16466540]
- Levin ER, Pietras RJ. Estrogen receptors outside the nucleus in breast cancer. *Breast Cancer Res. Treat.* 2007
- Li X, Zhang S, Safe S. Activation of kinase pathways in MCF-7 cells by 17beta-estradiol and structurally diverse estrogenic compounds. *J. Steroid Biochem. Mol. Biol* 2006;98:122–132. [PubMed: 16413991]
- Liu Z, Yu X, Shaikh ZA. Rapid activation of ERK1/2 and AKT in human breast cancer cells by cadmium. *Toxicol. Appl. Pharmacol* 2008;228:286–294. [PubMed: 18275979]
- Martin LA, Farmer I, Johnston SR, Ali S, Dowsett M. Elevated ERK1/ERK2/estrogen receptor cross-talk enhances estrogen-mediated signaling during long-term estrogen deprivation. *Endocr. Relat. Cancer* 2005;12(Suppl 1):S75–84. [PubMed: 16113101]
- Martin P, Poggi MC, Chambard JC, Boulukos KE, Pognonec P. Low dose cadmium poisoning results in sustained ERK phosphorylation and caspase activation. *Biochem. Biophys. Res. Commun* 2006;350:803–807. [PubMed: 17027645]
- Meyer MR, Haas E, Barton M. Gender differences of cardiovascular disease: new perspectives for estrogen receptor signaling. *Hypertension* 2006;47:1019–1026. [PubMed: 16651458]
- Nesatyy VJ, Ammann AA, Rutishauser BV, Suter MJ. Effect of cadmium on the interaction of 17beta-estradiol with the rainbow trout estrogen receptor. *Environ. Sci. Technol* 2006;40:1358–1363. [PubMed: 16572797]
- NHANES. Laboratory Assessment: Lab 6—Blood Lead and Cadmium, Blood Total Mercury, and Blood Inorganic Mercury. National Health and Nutrition Examination Survey 2003–2004 Data Documentation. 2006
- Pillon A, Boussioux AM, Escande A, Ait-Aissa S, Gomez E, Fenet H, Ruff M, Moras D, Vignon F, Duchesne MJ, Casellas C, Nicolas JC, Balaguer P. Binding of estrogenic compounds to recombinant estrogen receptor-alpha: application to environmental analysis. *Environ. Health Perspect* 2005;113:278–284. [PubMed: 15743715]
- Reddy KB, Yee D, Hilsenbeck SG, Coffey RJ, Osborne CK. Inhibition of estrogen-induced breast cancer cell proliferation by reduction in autocrine transforming growth factor alpha expression. *Cell Growth Differ* 1994;5:1275–1282. [PubMed: 7696176]

- Santen RJ, Song RX, McPherson R, Kumar R, Adam L, Jeng MH, Yue W. The role of mitogen-activated protein (MAP) kinase in breast cancer. *J. Steroid Biochem. Mol. Biol* 2002;80:239–256. [PubMed: 11897507]
- Schafer TE, Lapp CA, Hanes CM, Lewis JB, Wataha JC, Schuster GS. Estrogenicity of bisphenol A and bisphenol A dimethacrylate in vitro. *J. Biomed. Mater. Res* 1999;45:192–197. [PubMed: 10397975]
- Silva E, Lopez-Espinosa MJ, Molina-Molina JM, Fernandez M, Olea N, Kortenkamp A. Lack of activity of cadmium in in vitro estrogenicity assays. *Toxicol. Appl. Pharmacol* 2006;216(1):20–28. [PubMed: 16716372]
- Song, RXD.; McPherson, RA.; Adam, L.; Bao, Y.; Shupnik, M.; Kumar, R.; Santen, RJ. Linkage of rapid estrogen action to MAPK activation by ER{alpha}-Shc association and Shc pathway activation. 2002. p. 116-127.
- Stabile LP, Siegfried JM. Estrogen receptor pathways in lung cancer. *Curr. Oncol. Rep* 2004;6:259–267. [PubMed: 15161576]
- Stoica A, Katzenellenbogen BS, Martin MB. Activation of estrogen receptor-alpha by the heavy metal cadmium. *Mol. Endocrinol* 2000a;14:545–553. [PubMed: 10770491]
- Stoica A, Pentecost E, Martin MB. Effects of arsenite on estrogen receptor-alpha expression and activity in MCF-7 breast cancer cells. *Endocrinology* 2000b;141:3595–3602. [PubMed: 11014213]
- Waalkes MP. Cadmium carcinogenesis. *Mutat. Res* 2003;533:107–120. [PubMed: 14643415]
- Wilson VS, Bobseine K, Gray LE Jr. Development and characterization of a cell line that stably expresses an estrogen-responsive luciferase reporter for the detection of estrogen receptor agonist and antagonists. *Toxicol. Sci* 2004;81:69–77. [PubMed: 15166400]
- Yager JD, Davidson NE. Estrogen carcinogenesis in breast cancer. *N. Engl. J. Med* 2006;354:270–282. [PubMed: 16421368]
- Zivadinovic D, Watson CS. Membrane estrogen receptor-alpha levels predict estrogen-induced ERK1/2 activation in MCF-7 cells. *Breast Cancer Res* 2005;7:R130–R144. [PubMed: 15642162]

Fig. 1.

Effects of Cd on DNA synthesis. DNA synthesis was measured by BrdU incorporation. BrdU labeling index is the % BrdU labeled nuclei, which was routinely approximately 4% in controls (Ctrl). The concentrations are represented in nM. ER antagonist ICI 182,780 (ICI, final concentration 10 nM) was added 30 min before E2 where indicated. Results are presented as the mean and SD of three independent experiments. *Significantly greater than the control, $p < 0.01$.

Fig. 2.

Effects of Cd on PgR mRNA levels. (A) Cells were treated with 10 nM E2 for the times indicated, and PgR mRNA levels were determined by real time RT-PCR. (B) PgR mRNA levels in T47D cells treated with different concentrations (nM) of Cd or E2 for 20 h. Results are presented as mean and SD of three independent experiments. *Significantly ($p < 0.01$) higher than the control.

Fig. 3.

Effects of Cd on the early phosphorylation of ERK1/2 MAPK, measured after 10 min of treatment. E2 was included as a positive control. Cells in the control (Ctrl) group were similarly treated with PBS. The western blot picture is representative of three treatments. The ratio of band intensity of phosphorylated ERK1/2 (p-ERK) to that of total ERK1/2 (t-ERK) was measured by the LI-COR Odyssey Infrared Imaging System and analyzed using Odyssey software. Each bar represents the mean and SD of three experiments. *Significantly different from the control, $p < 0.01$.

Fig. 4.

Effects of Cd on the phosphorylation of ERK1/2 MAPK, measured after 18 h of treatment. (A) Dose response of ERK1/2 activation by Cd following an 18-h exposure. (B) Cd-induced late ERK1/2 phosphorylation in the presence of 10 nM ICI 182,780 (added 30 min before Cd). The left most lane serves as a no-ICI control. (C) The ratio of the band intensity of p-ERK to that of t-ERK in absence or presence of ICI 182,780. Each bar represents the mean and SD of three (without ICI 182,780) or range of duplicate (with ICI) experiments. *Significantly different from the control, $p < 0.01$.

Fig. 5.

Effects of Cd on DNA synthesis in the presence of 10 pM E2, with or without the MEK1/2 inhibitor U0126. DNA synthesis was measured by BrdU incorporation. The top left insert is a dose response curve of E2-induced DNA synthesis showing that 10 pM E2 corresponds to its EC_{20} for this induction. Cd was added 30 min prior to E2 and cells were treated with the Cd–E2 mixture for 48 h. In groups with MEK1/2 inhibitor U0126, U0126 (final concentration: 10 μ M) was added 30 min before Cd and remained in the media throughout the treatment. Results are presented as mean and SD of three independent experiments. *Significantly ($p < 0.01$) higher than the E2-only control.

Fig. 6. Effects of Cd on pS2 and PgR gene expression in the absence or presence of 10 pM E2. Cells were treated for 24 h. Results are presented as mean and SD of three independent experiments. *Significantly different ($p < 0.05$); NS: not significant.