— **Minireview** —

Interaction between Dioxin Signaling and Sex Steroid Hormones

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Dioxin, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), is an environmental contaminant that produces potent toxic effects in humans and animals. TCDD is carcinogenic at multiorgan sites and induces disorders of reproduction, development, and immunity. It has been considered that the effects of TCDD partly involve disruption of the endocrine system, since TCDD causes progressive endometriosis in the rhesus monkey, suppresses development of the male reproductive system and sexual dimorphism of the brain, and damages the ovaries. These effects suggest alteration of sex steroidogenesis in the target organs. To clarify the endocrine disruptive action of TCDD, many studies of its effects on estrogen-responsive cell lines have been conducted. This mini-review describes recent experiments conducted in the authors' laboratory and discusses our understanding of the molecular mechanisms of interaction between dioxin signaling and sex-steroid hormones, focusing on three issues: 1) influence of estrogen on TCDD-induced cytochrome P4501A1 (CYP1A1) protein *in vivo*; 2) influence of estrogen on TCDD-induced xenobiotic response element (XRE) transactivation in cultured cell systems; and 3) effect of hormone-receptor status on TCDD-induced XRE transactivation and its relation to the cell cycle.

Key words —— human cells, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, cytochrome P4501A1, aryl hydrocarbon receptor, estrogen receptor, steroid hormone

INTRODUCTION

The so-called dioxin-like family of compounds comprises a number of chemical classes, including polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans, polybrominated dibenzo-*p*-dioxins, polybrominated dibenzofurans, and polychlorinated biphenyls. Among them, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, dioxin) is the prototype and most toxic member. TCDD has various toxic effects including carcinogenicity, hepatotoxicity, immunotoxicity, reproductive and developmental toxicity, and neurotoxicity.[1,2] These toxic effects vary according to sex, age, species, and strain of experimental animals. Administration of TCDD in the diet to rats of both sexes for a period of 2 years has been reported to induce neoplasms in the liver and skin only of females, but to reduce the development of mammary tumors, suggesting that TCDD exhibits conflicting actions in hormone-dependent tissues.[3,4] Similarly, *in utero* and lactational exposure to TCDD has been reported to produce abnormalities in female pups, such as the presence of vaginal threads, delayed vaginal opening, and retarded development of the mammary epithelium.[5,6] These effects appear to be linked to the actions of sex steroid hormones including estrogen, testosterone, and progesterone and suggest that TCDD blocks the action of the estrogen receptor (ER).

It is considered that the effects of TCDD are generally mediated via the aryl hydrocarbon receptor (AhR) signaling pathway (Fig. 1).[7] Typical biochemical responses to TCDD include induction of cytochrome P450 (CYP) isozymes and the metabolizing enzymes of several other drugs, cytokines, and various other growth factors.[7,8] The molecular mechanism of action of TCDD is basically similar to that proposed for the intracellular actions of steroid hormones.[9] The AhR and steroid hormone re-

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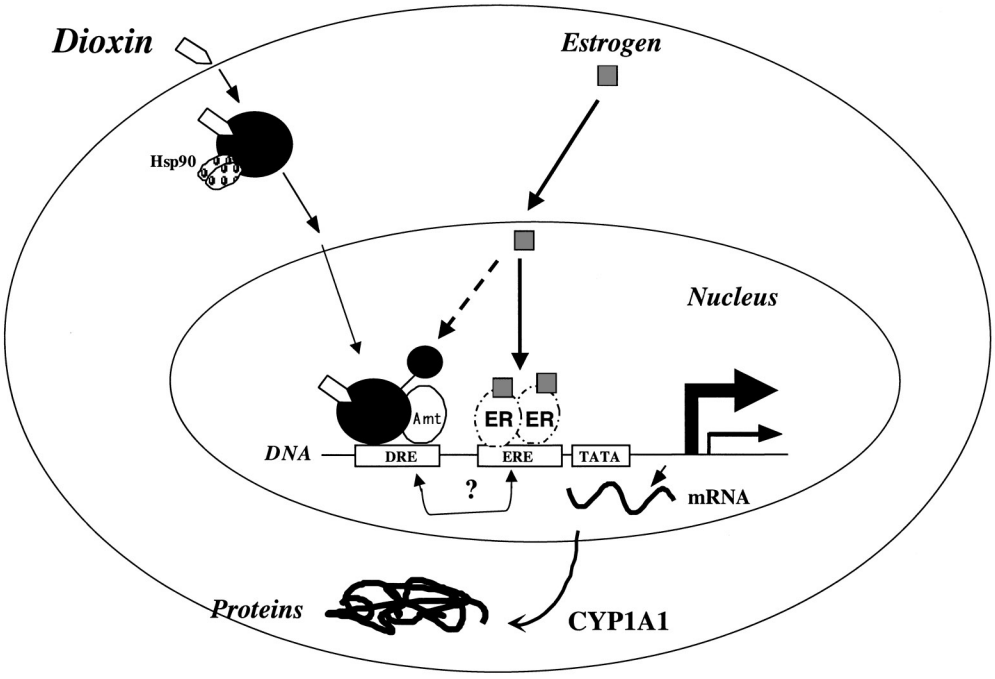

Fig. 1. Molecular Mechanisms of Dioxin and AhR Signaling
The biological effects of dioxin are mediated through AhR pathways (see Introduction).

ceptors are ligand-activated transcription factors and members of the basic helix-loop-helix superfamily of DNA binding proteins. The liganded AhR translocates to the nucleus, followed by dimerization to the aryl hydrocarbon receptor nuclear translocator (ARNT).[10] Once inside the nucleus, the AhR-ARNT heterodimer binds with specific *cis*-acting enhancers known as xenobiotic or dioxin response elements (XREs or DREs), which promote the activation of a battery of numerous genes. Up to now, there has been a general consensus that subsequent recruitment of coactivators and general transcription factors modifies the transactivation of Ah-responsive genes. Among these induced genes, expression of the cytochrome P4501A1 (CYP1A1) gene is the most sensitive and earliest biochemical response and is therefore used as a response marker gene for TCDD.[11,12]

Many important studies have demonstrated inhibitory AhR-ER crosstalk in the rodent uterus and mammary gland, and also in human breast cancer cells. In human breast cancer cell lines with or without ER, TCDD inhibits 17β-estradiol (E2)-dependent cell proliferation[13] and secretion of E2-induced proteins such as tissue plasminogen activator, cathepsin-D, and pS2.[14] None of these effects have been found in ER-negative breast cancer cells.[15] There is substantial evidence that TCDD does not interact directly with the ER or progesterone receptor (PR),[16] and therefore the antisteroidogenic effects of TCDD cannot be explained by direct interaction with those receptors, but rather by a decrease in the number of receptors through inhibition of steroid-induced gene transcription.[17] Its interference with transcription has been explained by possible competition between steroid hormone receptors and the liganded AhR-ARNT complex for XREs on steroid-induced genes.[18,19]

In the present mini-review, we describe recent studies on the interaction between TCDD-induced gene expression and estrogen *in vivo* and in hormone-dependent cell lines in an attempt to clarify the molecular mechanisms responsible for the disruptive action of TCDD on hormonal signals.[20–22]

Influence of Estrogen on TCDD-Induced CYP1A1 Protein *in Vivo*

Expression of CYP1A1 protein and ethoxyresorufin-*O*-deethylase (EROD) activity in liver was induced in both intact and ovariectomized (OVX) rats treated with a single oral dose of 300 ng TCDD/kg, and the level of expression increased when rats were given a combination of TCDD and E2 (Fig. 2A and B). The EROD activity in rats treated with both TCDD and E2 was significantly higher than that in rats treated with TCDD alone, suggesting that E2 enhances TCDD-induced CYP1A1. In contrast, no CYP1A1 protein or its activity was detected in the

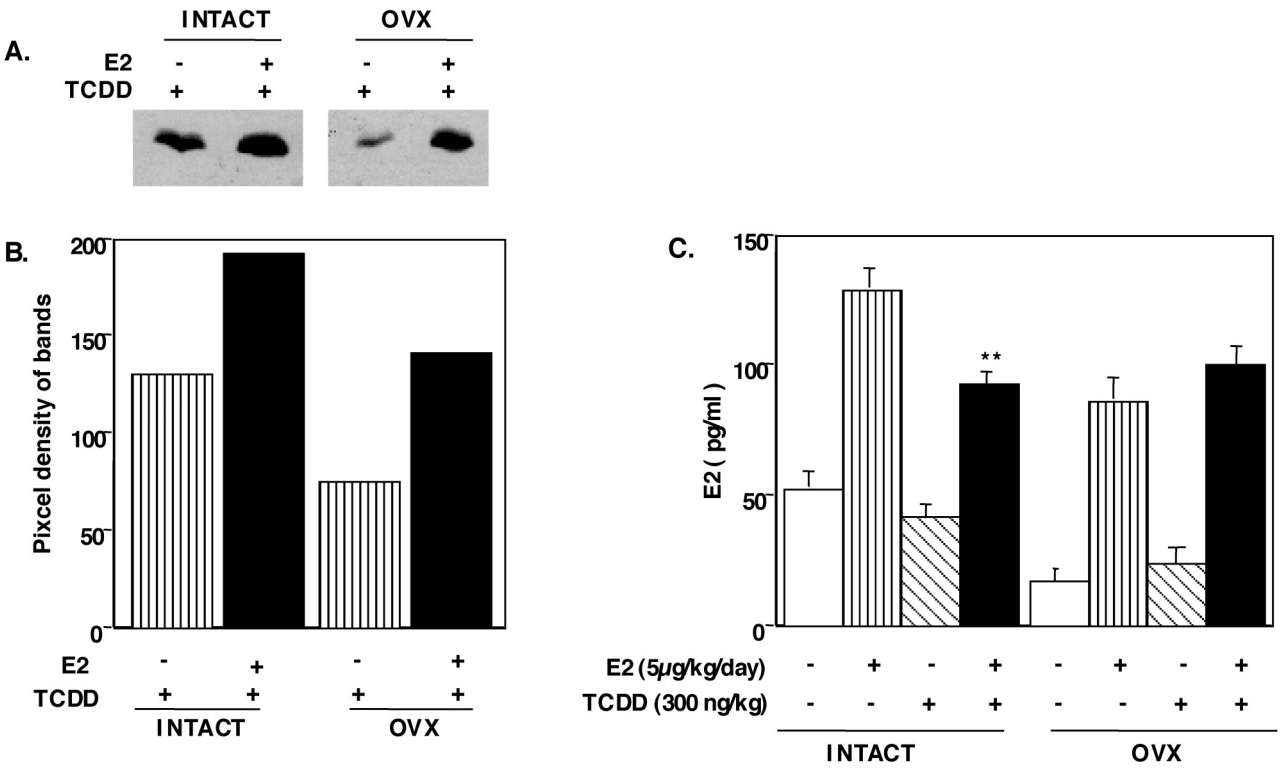

Fig. 2. Induction of CYP1A1 Protein by TCDD in the Liver Microsomal Fraction of Intact and OVX Rats

(A) Representative immunoblots. (B) Quantitative change in CYP1A1 expression. The values depicted are the mean pixel density of each band in three assays. (C) Effect of TCDD (300 ng/kg) and estrogen (5 μg/kg) on circulating E2 in intact and OVX rats. Values are mean ± S.D. from 3–4 animals in each group. ** Statistical significance at $p < 0.001$ compared with intact rats treated with estrogen alone or in combination with TCDD.

hepatic microsomal fractions of rats that were not treated with TCDD. Data on EROD activity and plasma E2 level in intact and OVX rats were subjected to linear fit analysis and the correlation coefficients were determined. A positive correlation between plasma E2 level and EROD activity induced by TCDD was observed at coefficient values of 0.876 and 0.891 in intact and OVX rats, respectively. Thus it was demonstrated that a relationship exists between TCDD-induced EROD activity and estrogen levels *in vivo*, and the present observations show that TCDD-induced CYP1A1 expression was enhanced by estrogen treatment *in vivo*. It is suggested that estrogen plays a role in transactivation of the CYP1A1 gene in response to TCDD stimulation.

It is well known that CYP1A1 enzyme function oxygenates chemical carcinogens such as polycyclic aromatic hydrocarbons (PAH) and then generates arene oxides that can produce mutations of oncogenes leading to neoplasms. Therefore such enhancement would modify the development of liver cancer in female rats.

Influence of Estrogen on TCDD-Induced XRE Transactivation in a Cultured Cell System

The actions of estrogen are mediated through estrogen receptor alpha (ER-α), and defects of ER-α result in various forms of reproductive dysfunction in female mice.[23] The next study was conducted to investigate the role of ER-α in the TCDD responsiveness of human uterine endometrial carcinoma cells, RL95-2 and KLE. RL95-2 cells were highly responsive to TCDD in terms of CYP1A1, cytochrome P4501B1 (CYP1B1), and plasminogen activator inhibitor-2 (PAI-2) induction, whereas KLE cells were responsive only at high doses. Neither showed any growth inhibition upon exposure to TCDD. KLE cells expressed higher levels of AhR than RL95-2 cells, and gel mobility shift assay also identified more liganded AhR-ARNT complex bound to XRE. TCDD had no down-regulatory effects on the expression of either AhR or the ER in both cell lines. Although both cell types expressed ER-α almost equally, immunofluorescence demonstrated a defect in its nuclear translocation in KLE cells, where ER-α was mainly cytoplasmic and E2 was unable to translocate it to the nucleus. How-

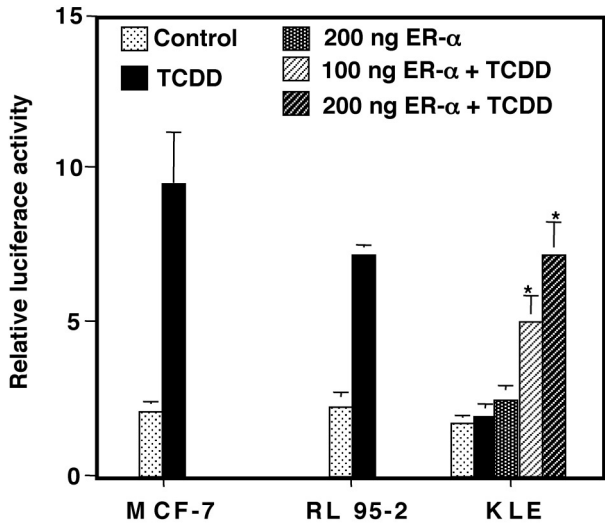

Fig. 3. Effects of ER-α on TCDD Responsiveness in RL95-2 and KLE Cells

Cells were grown in steroid-free medium and cotransfected with the TCDD-responsive reporter plasmid pGL3-1(XRE)-LUC, an internal control plasmid pRL-SV40, and increasing amounts of the ER-α expression plasmid, HEO (100–200 ng/well). After transfection, the cells were treated with either 0.1% DMSO or 10 nM TCDD for 24 hr and then processed for dual luciferase reporter assays. Results are means ± S.D. of two independent experiments each performed in triplicate. MCF-7 cells were used as a positive control.

ever, both cell types were nonresponsive to E2 in terms of transcriptional activation, and transient expression of normal ER-α restored the E2 responsiveness. Transient expression of ER-α in KLE cells also restored its responsiveness to TCDD on transcriptional activation. Furthermore, to test whether the defective ER-α, and therefore the blocked nuclear translocation, in KLE cells was the main cause of their minimal responsiveness to TCDD, transient transfection with a normal ER-α expression plasmid along with a TCDD-responsive reporter plasmid, pGL3-1(XRE)-LUC, was performed. As shown in Fig. 3, transient transfection with pGL3-1(XRE)-LUC and subsequent treatment with 10 nM TCDD increased reporter gene activity several-fold in MCF-7 and RL95-2 cells, but not in KLE cells. However, cotransfection of ER-α with pGL3-1 (XRE)-LUC and subsequent TCDD (10 nM) treatment significantly increased the reporter gene activity and this was positively correlated with the amount of ER used in the transfection. These results indicate that ER-α acts as a positive modulator in the regulation of TCDD-inducible genes and is necessary for transactivation of genes mediated through XRE.

In human breast cancer cell lines, induction of CYP1A1 appears to be related to their ER-α content,[24] and Ah responsiveness is dependent not only on the expression of AhR but also ER-α levels.[24–26] ER-negative breast cancer cell lines such as MDA-MB-231 and Hs578T are normally not responsive to Ah, but transient transfection of ER-α into these cells restores their Ah responsiveness.[25,26] Detailed sequential reporter gene assays have revealed that both the *N*- and *C*-terminal transactivation domains of ER-α are responsible for AhR responsiveness.[26] The mechanisms involved are unclear, but several possibilities can be speculated: 1) ER-α might interact with liganded AhR-ARNT complexes directly or through some bridging factors; and 2) ER-α might displace negative regulatory factors or facilitate the binding of critical transcription factors to the upstream promoter region. One report has indicated that ER-α does not interact directly with the liganded AhR-ARNT complex,[27] but both of these can physically interact with Sp1 protein.[28,29] Whether this interaction really increases the transactivation potential of TCDD-inducible genes remains to be clarified.

Effect of Hormone Receptor Status on TCDD-Induced XRE Transactivation and its Relation to the Cell Cycle

Neither RL95-2 nor KLE cell lines exhibited any cell growth inhibition by TCDD, whereas the former proved highly responsive to TCDD in the induction of several genes, unlike the latter. This is consistent with another observation that TCDD induced up-regulation of interleukin-1β, urokinase plasminogen activator, and tumor necrosis factor-α.[30] TCDD has been shown to down-regulate epidermal growth factor receptor (EGFR) in reproductive tissues and also several cell lines.[31,32] A recent report has indicated that TCDD inhibits EGF withdrawal-induced apoptosis and increases cell growth in MCF-10A, an ER-negative cell line, suggesting that TCDD acts as tumor promoter in ER-negative cells.[33] In contrast, TCDD inhibits the growth of tumor-derived cell lines that overexpress ER.[34,35]

Therefore we determined the steady-state levels of the ER-α, ER-β, androgen receptor (AR), and PR genes in the tumor-derived cell lines MCF-7, RL95-2, and LNCaP (Fig. 4A). MCF-7 cells expressed a very high level of ER-α compared with RL95-2 and LNCaP. The expression level of ER-β was similar in RL95-2 and LNCaP cells. ER-α and AR were expressed in MCF-7 and LNCaP cells, but not in RL95-2 cells, whereas PR was expressed in MCF-7 and RL95-2 cells, but not in LNCaP cells. In all cases,

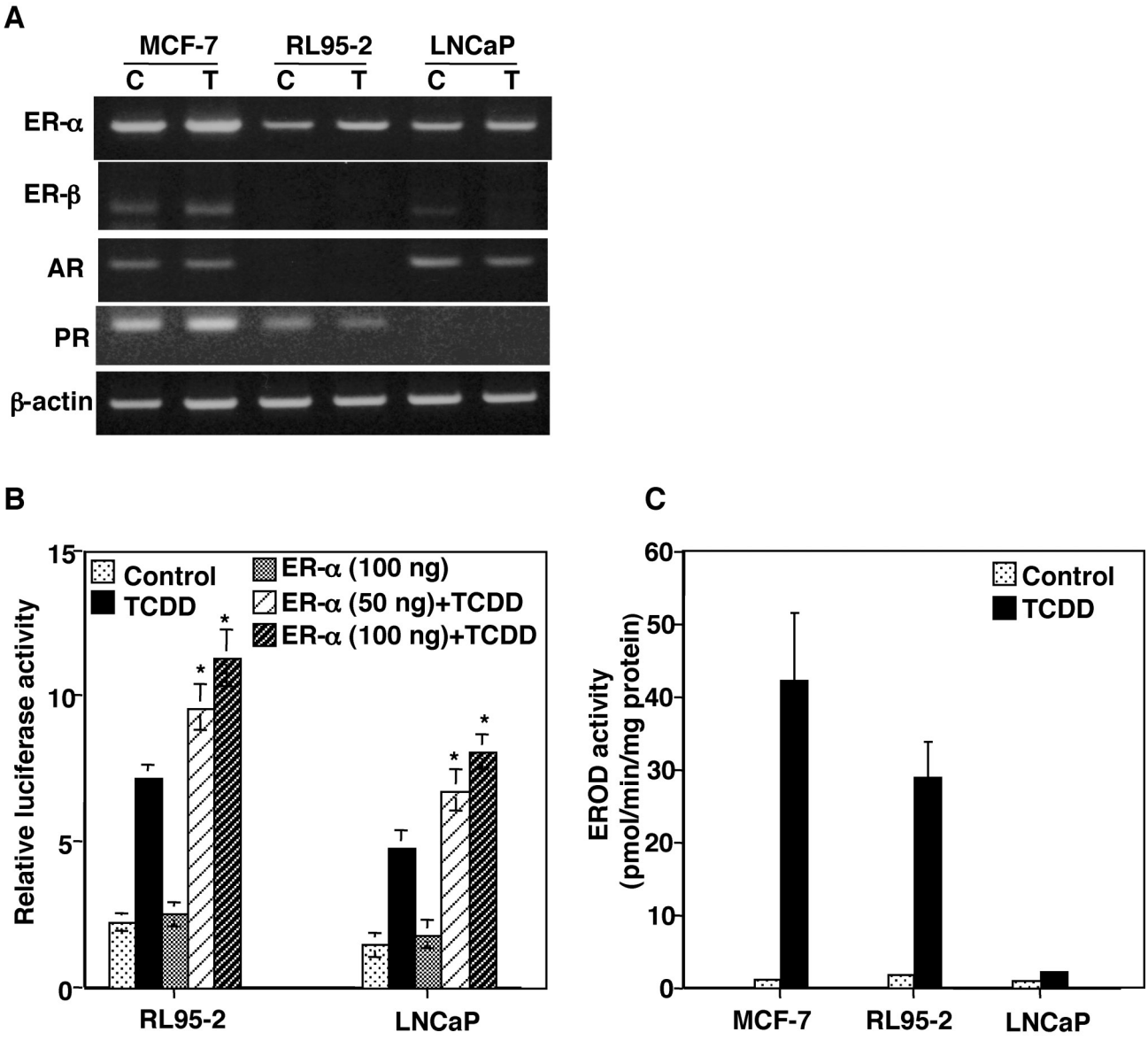

Fig. 4. Effect of Hormone-Receptor Status on AhR-Mediated Transcription in MCF-7, RL95-2, and LNCaP Cells

RT-PCR analysis of ER-α (438 bp), ER-β (220 bp), AR (203 bp), and PR (193 bp) mRNA transcripts in untreated (C) and TCDD-treated (T) cells (A). Effect of transient expression of ER-α on XRE-driven luciferase activity in RL95-2 and LNCaP (B). Effect of EROD activity in MCF-7, RL95-2 and LNCaP cells (C). Results are the mean ± S.D. The asterisk indicates a statistically significant difference, at $p < 0.01$, from the ER-α alone treatment group.

TCDD had almost no effect on the expression of these genes. Figure 4B shows similar levels of induction of TCDD-responsive reporter gene activity driven by a single XRE element in RL95-2 and LNCaP cells. These data are noteworthy because RL95-2 cells showed an approximately 10-fold increase in EROD activity compared with LNCaP cells (Fig. 4C). This could not be explained by ER-α content, since ER-α expression was similar, or possibly lower, in LNCaP cells, and thus it appears that some other factor(s) might be involved. In our search for likely factors, we found that DNA methylation in the CpG dinucleotide of the XRE core sequence might be a plausible candidate, since pretreatment with a DNA methyltransferase inhibitor, azacytidine, significantly increased EROD activity in LNCaP cells. However, azacytidine had no effect on MCF-7 or RL95-2 cells. This type of DNA methylation has been reported to be involved in the silencing of CYP1A1 gene expression in rabbit kidney cells.[36] We cannot rule out another possibility that AR in LNCaP may play some role in transcriptional activation mediated through AhR because a previous study has reported that LNCaP cells contain an AR

with a point mutation in the steroid-binding domain (codon 868, Thr to Ala), which leads to a change in specificity of the AR.[37]

Other studies of cell cycle checkpoints have suggested that AhR plays an important role in the regulation of cell growth and differentiation. In particular, TCDD has been shown to suppress transcriptional activation in G2/M cells mediated through AhR.[38] Overproduction of TGF-β in AhR-deficient cells appears to result in low proliferation rates and increased apoptosis, leading to an alteration in cell cycle control. Our most recent study has shown that TCDD affects transcriptional activation in different ways in two cell lines, BeWo and RL95-2, differing in cell cycle status, whereas both cell lines have similar ER contents. In human placental choriocarcinoma BeWo cells with a cell cycle status of G_0/G_1: 45%; S: 45%; G_2/M: 10%, TCDD increased the proportion of cells in S phase and reduced the proportion in G_0/G_1 phase. By contrast, in the G_0/G_1-predominant cell line RL95-2, TCDD increased the number of cells in the G_0/G_1-phase and reduced those in S-phase. However, TCDD increased the activity of human telomerase reverse transcriptase (hTERT), a regulatory subunit of telomerase, in BeWo cells but not in RL95-2 cells. These results suggest that TCDD exerts contrasting effects depending on cell-cycle status.

Thus TCDD acts like a partial agonist, and/or antagonist of ER, and E2, inhibits or enhances TCDD-responsive genes. To understand the molecular mechanisms involved in the broad spectrum of TCDD toxicity, further detailed studies will be required. For example, analysis of the promoter regions of TCDD- or estrogen-responsive genes would suggest how ligand-specific consensus sequences including the XRE, ERE, and LXXR motifs, and the AP1 or SP1 site, combinatorially regulate their transcription.

REFERENCES

1) Yonemoto, J. (2000) The effects of dioxin on reproduction and development. *Ind. Health*, **38**, 259–268.
2) Birnbaum, L. S. and Tuomisto, J. (2000) Noncarcinogenic effects of TCDD in animals. *Food Addit. Contam.*, **17**, 275–288.
3) I.A.R.C. (1997) Monograph on the Evaluation of Carcinogenic risk to Humans. In *Polychlorinated Dibenzo-para-Dioxins and Polychlorinated Dibenzofurans*, I.A.R.C. Press, Geneva, No. 69, pp. 137–195.
4) Kociba, R. J., Keyes, D. G., Beyer, J. E., Carreon, R. M., Wade, C. E., Dittenbe D. A., Kalnins, R. P., Frauson, L. E., Park, N., Barnard, S. D., Hummel, R. A. and Humiston, C. G. (1978) Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rats. *Toxicol. Appl. Pharmacol.*, **46**, 279–303.
5) Flaws, J. A., Sommer, R. J., Silbergeld, E. K., Peterson, R. E. and Hirshfield, A. N. (1997) *In utero* and lactational exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) induces genital dysmorphogenesis in the female rat. *Toxicol. Appl. Pharmacol.*, **147**, 351–362.
6) Gray, L. E., Jr. and Ostby, J. S. (1995) *In utero* 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) alters reproductive morphology and function in female rat offspring. *Toxicol. Appl. Pharmacol.*, **133**, 285–294.
7) Poland, A. and Knutson, J. C. (1982) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. *Annu. Rev. Pharmacol. Toxicol.*, **22**, 517–554.
8) Davis, J. W., II, Lauer, F. T., Burdickm A. D., Hudson, L. and Burchiel, S. W. (2001) Prevention of apoptosis by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the MCF-10A cell line: correlation with increased transforming growth factor alpha production. *Cancer Res.*, **61**, 3314–3320.
9) Safe, S., Wang, F., Porter, W., Duan, R. and McDougal, A. (1998) Ah receptor agonists as endocrine disruptors: antiestrogenic activity and mechanisms. *Toxicol. Lett.*, **102–103**, 343–347.
10) Hankinson, O. (1995) The aryl hydrocarbon receptor complex. *Annu. Rev. Pharmacol. Toxicol.*, **35**, 307–340.
11) Reyes, H., Reisz-Porszasz, S. and Hankinson, O. (1992) Identification of the Ah receptor nuclear translocator protein (Arnt) as a component of the DNA binding form of the Ah receptor. *Science*, **256**, 1193–1195.
12) Burbach, M., Poalnd, A. and Bradfield, C. A. (1992) Cloning of the Ah receptor C-DNA reveals a distincitive ligand activated transcription factor. *Proc. Natl. Acad. Sci. U.S.A.*, **89**, 8185–8189.
13) Gierthy, J. F., Lincoln, D. W., Gillespie, M. B., Seeger, J. I., Martinez, H. L., Dickerman, H. W. and Kumar, S. A. (1987) Suppression of estrogen-regulated extracellular tissue plasminogen activator activity of MCF-7 cells by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Cancer Res.*, **47**, 6198–6203.
14) Spink, D. C., Lincoln, D. W., Dickerman, H. W. and Gierthy, J. F. (1990) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin causes an extensive alteration of 17 beta-estradiol metabolism in MCF-7 breast tumor cells.

Proc. Natl. Acad. Sci. U.S.A., **87**, 6917–6921.
15) Klinge, C. M., Bowers, J. L., Kulakosky, P. C., Kamboj, K. K. and Swanson, H. I. (1999) The aryl hydrocarbon receptor (AHR)/AHR nuclear translocator (ARNT) heterodimer interacts with naturally occurring estrogen response elements. *Mol. Cell Endocrinol.*, **157**, 105–119.
16) Dohr, O., Vogel, C. and Abel, J. (1995) Different response of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-sensitive genes in human breast cancer MCF-7 and MDA-MB 231 cells. *Arch. Biochem. Biophys.*, **321**, 405–412.
17) Romkes, M. and Safe, S. (1988) Comparative activities of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and progesterone as antiestrogens in the female rat uterus. *Toxicol. Appl. Pharmacol.*, **92**, 368–380.
18) Hoivik, D., Willett, K., Wilson, C. and Safe, S. (1997) Estrogen does not inhibit 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-mediated effects in MCF-7 and Hepa 1c1c7 cells. *J. Biol. Chem.*, **272**, 30270–30274.
19) Kharat, I. and Saatcioglu, F. (1996) Antiestrogenic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin are mediated by direct transcriptional interference with the liganded estrogen receptor: Cross-talk between aryl hydrocarbon- and estrogen-mediated signaling. *J. Biol. Chem.*, **271**, 10533–10537.
20) Sarkar, S., Jana, N. R., Yonemoto, J., Tohyama, C. and Sone, H. (2000) Estrogen enhances induction of cytochrome P-4501A1 by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in liver of female Long-Evans rats. *Int. J. Oncol.*, **16**, 141–147.
21) Jana, N. R., Sarkar, S., Ishizuka, M., Yonemoto, J., Tohyama, C. and Sone, H. (1999) Role of estradiol receptor-α in differential expression of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-inducible genes in the RL95-2 and KLE human endometrial cancer cell lines. *Arch. Biochem. Biophys.*, **368**, 31–39.
22) Jana, N. R., Sarkar, S., Ishizuka, M., Yonemoto, J., Tohyama, C. and Sone, H. (2000) Comparative effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on MCF-7, RL95-2, and LNCaP cells: role of target steroid hormones in cellular responsiveness to CYP1A1 induction. *Mol. Cell Biol. Res. Commun.*, **4**, 174–180.
23) Course, J. F. and Korach, K. S. (2001) Contrasting phenotypes in reproductive tissues of female estrogen receptor null mice. *Ann. N.Y. Acad. Sci.*, **948**, 1–8.
24) Vickers, P. J., Dufresne, M. J. and Cowan, K. H. (1989) Relation between cytochrome P450IA1 expression and estrogen receptor content of human breast cancer cells. *Mol. Endocrinol.*, **3**, 157–164.
25) Thomsen, J. S., Wang, X., Hines, R. N. and Safe, S. (1994) Restoration of aryl hydrocarbon (Ah) responsiveness in MDA-MB-231 human breast cancer cells by transient expression of the estrogen receptor. *Carcinogenesis*, **15**, 933–937.
26) Wang, W. L., Thomsen, J. S., Porter, W., Moore, M. and Safe, S. (1996) Effect of transient expression of the oestrogen receptor on constitutive and inducible CYP1A1 in Hs578T human breast cancer cells. *Br. J. Cancer*, **73**, 316–322.
27) Miller, C. A., Martinat, M. A. and Hyman, L. E. (1998) Assessment of aryl hydrocarbon receptor complex interactions using pBEVY plasmids: expression vectors with bi-directional promoters for use in *Saccharomyces cerevisiae*. *Nucleic Acids Res.*, **26**, 3577–3583.
28) Kobayashi, A., Sogawa, K. and Fujii-Kuriyama, Y. (1996) Cooperative interaction between AhR.Arnt and Sp1 for the drug-inducible expression of CYP1A1 gene. *J. Biol. Chem.*, **271**, 12310–12316.
29) Wang, F., Hoivik, D., Pollenz, R. and Safe, S. (1998) Functional and physical interactions between the estrogen receptor Sp1 and nuclear aryl hydrocarbon receptor complexes. *Nucleic Acids Res.*, **26**, 3044–3052.
30) Charles, G. D. and Shiverick, K. T. (1997) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin increases mRNA levels for interleukin-1beta, urokinase plasminogen activator, and tumor necrosis factor-alpha in human uterine endometrial adenocarcinoma RL95-2 cells. *Biochem. Biophys. Res. Commun.*, **238**, 338–342.
31) Sewall, C. H., Clark, G. C. and Lucier, G. W. (1996) TCDD reduces rat hepatic epidermal growth factor receptor: comparison of binding, immunodetection, and autophosphorylation. *Toxicol. Appl. Pharmacol.*, **132**, 263–272.
32) Enan, E., Moran, F., VandeVoort, C. A., Stewart, D. R., Overstreet, J. W. and Lasley, B. L. (1996) Mechanism of toxic action of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in cultured human luteinized granulosa cells. *Reprod. Toxicol.*, **10**, 497–508.
33) Davis, J. W., II, Lauer, F. T., Burdick, A. D., Hudson, L. G. and Burchiel, S. W. (2001) Prevention of apoptosis by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the MCF-10A cell line: correlation with increased transforming growth factor alpha production. *Cancer Res.*, **61**, 3314–3320.
34) Fernandez, P. and Safe, S. (1992) Growth inhibitory and antimitogenic activity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in T47D human breast cancer cells. *Toxicol. Lett.*, **61**, 185–197.
35) Liu, H. and Safe, S. (1996) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on insulin-induced responses in MCF-7 human breast cancer cells. *Toxicol. Appl. Pharmacol.*, **138**, 242–250.
36) Takahashi, Y., Suzuki, C. and Kamataki, T. (1998) Silencing of CYP1A1 expression in rabbits by DNA

methylation. *Biochem. Biophys. Res. Commun.*, **247**, 383–386.
37) Veldscholte, J., Berrevoets, C. A., Brinkmann, A. O., Grootegoed, J. A. and Mulder, E. (1992) Anti-androgens and the mutated androgen receptor of LNCaP cells: differential effects on binding affinity, heat-shock protein interaction, and transcription activation. *Biochemistry*, **31**, 2393–2399.
38) Elizondo, G., Fernandez-Salguero, P., Sheikh, M. S., Kim, G. Y., Fornace, A. J., Lee, K. S. and Gonzalez, F. J. (2000) Altered cell cycle control at the G_2/M phases in aryl hydrocarbon receptor-null embryo fibroblast. *Mol. Pharmacol.*, **57**, 1056–1063.