A Novel Mechanism for Restoring Aryl Hydrocarbon (Ah) Responsiveness in Hs578T Human Breast Cancer Cells

W. L. Wang, J. S. Thomsen, W. Porter, M. Moore and S. Safe
Departments of Veterinary Physiology and Pharmacology and Biochemistry/Biophysics, Texas A&M University, College Station, TX 77843-4466

1. Introduction

Vickers and coworkers [1] have suggested that induction of CYP1A1 in human breast cancer cells is related to their estrogen receptor (ER) content and studies with several different human breast cancer lines indicate that Ah-responsiveness correlates with expression of both the ER and aryl hydrocarbon receptor (AhR). Moreover, several cell lines which express the AhR but are ER-negative are not Ah-responsive and these include MDA-MB-231, Hs578T and adriamycin-resistant MCF-7 breast cancer cells [1-7]. A recent study from this laboratory [3] showed that chloramphenicol acetyl transferase (CAT) activity was induced by TCDD in MDA-MB-231 cells transiently transfected with the human ER (hER) expression plasmid and pRNH11c, an Ah-responsive plasmid containing a dioxin responsive element (DRE) derived from the 5'-regulatory region of the human CYP1A1 gene. The role of the ER in restoring Ah-responsiveness in ER-negative human breast cancer cells was investigated using the Hs578T cell line as a model. Hs578T cells expressed the AhR; however, TCDD did not induce CYP1A1 gene expression. In transient transfection studies with the hER expression and pRNH11c plasmids, there was a significant increase in basal but not TCDD-induced CAT activity. Although the full length hER did not restore Ah-responsiveness in Hs578T cells, cotransfection with an N-terminal truncated hER construct resulted in restoration of Ah-responsiveness in this cell line.

2. Materials and Methods

Chemicals and Biochemicals. TCDD and 2,3,7,8-tetrachlorodibenzofuran (TCDF) (> 99% pure) was prepared in this laboratory. [^{3}H]TCDD (37 Ci/mmol) was prepared in this laboratory and purified by high-pressure liquid chromatography (> 98% pure). All other chemicals and biochemicals were the highest quality available from commercial sources.

Cell Culture Maintenance and Growth. The Hs578T human breast cancer cells were obtained from the America Type Culture Collection and maintained in DME/F12 medium with phenol red and supplemented with 5% fetal bovine serum plus 10 ml antibiotic/antimycotic solution at 37°C.

Expression Plasmids. The plasmid pRNH11c was kindly provided by Dr. R. Hines and

contains the regulatory human CYP1A1 region from the TaqI site at -1142 to the BclI site at +2434 fused to the bacterial CAT reporter gene [8]. The hER plasmid was generous gift from Dr. Ming Jer Tsai (Baylor College of Medicine). This plasmid contains the human estrogen receptor cDNA. HE15 and HE19 are expression vectors coding for mutant human ERs and were kindly provided by Dr. P. Chambon (Strasbourg, France). In HE15, the amino acids from 282 to 595 are deleted, whereas HE19 is truncated from amino acids 1 to 178. Arnt and AhR cDNAs were kindly provided by Drs. Bradfield and Hankinson [9,10] and constructed into pcDNA1 and pcDNA3 vectors, respectively.

Transient Transfection Assay. Cells were seeded in 100 mm Petri dishes and grown until 70% confluent, 5 to 10 μg of each plasmid and 20 μg polybrene/ml were used for each assay. After incubation for 6 h, cells were shocked using 25% DMSO. After 18 h, cells were treated with DMSO (0.2% total volume) or TCDD (10 nM) in DMSO for 44 h. Cells were then washed with PBS and scraped from the plates. Cell lysates were prepared in 0.16 ml of 0.25M Tris-HCl, pH 7.5, by three freeze-thaw-sonication cycles (3 min/each). CAT activity was determined using 0.2 mCi d-threo-[dichloroacetyl-1-^{14}C]chloramphenicol and 4 mM acetyl-CoA as substrates. Following TLC, acetylated products were visualized and quantitated using a Betascope 603 Blot analyzer as previously described.

3. Results and Discussion

The results illustrated in Figure 1 summarize the effects of DMSO (lanes 1, 3, 5, 7, 9 and 11) or 10 nM TCDD (2, 4, 6, 8, 10 and 12) on induction of CAT activity in Hs578T cell transiently transfected with pRNH11c in the presence of combinations of Arnt, AhR and hER expression plasmids. Transfection of Arnt, AhR or Arnt plus AhR expression plasmids does not significantly affect basal or TCDD-inducible CAT activity in Hs578T cells. In contrast, cotransfection of hER

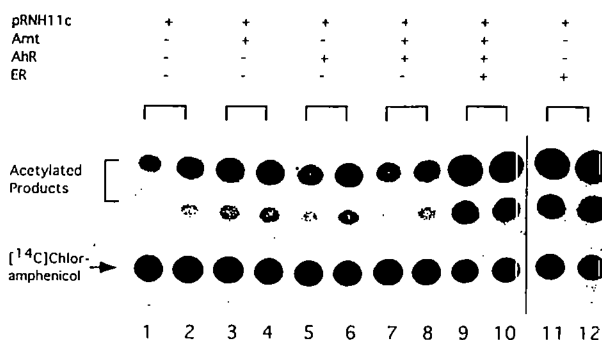

Figure 1. Effects of human AhR, Arnt and ER on restoration of Ah-responsiveness in Hs578T human breast cancer cells. The cells were transiently transfected with 5μg of pRNH11c (lanes 1 through 12) and cotransfected with 5 μg of other expression plasmids for each different experiments. Lanes 1, 3, 5, 7, 9 and 11 were derived from cells treated with DMSO, whereas lanes 2, 4, 6, 8, 10 and 12 were treated with 10 nM TCDD for 44 h.

Table 1. Comparative effects of human wild- and mutant-type ER expression plasmids on restoring Ah-responsiveness to Hs578T cells by transient cotransfection studies with Ah-responsive pRNH11c plasmid.*

	Relative CAT Activity	
Transfected Plasmids	DMSO	TCDD
pRNH11c + hER	100	151.9[a]
pRNH11c + HE15	45.9[a]	51.9[a]
pRNH11c + HE19	2.1[a]	48.9[a]

* The cells were transfected with 5 μg of each plasmid for each individual group, shocked with 25% DMSO, and dosed with DMSO or 10 nM TCDD for 44 h and standardized against DMSO-treated Hs578T cells which were cotransfected with pRNH11c plasmid alone and the full length or truncated ER expression plasmids.

[a] Statistically higher ($p < 0.01$) than DMSO-treated Hs578T cells cotransfected with pRNH11c plus ER plasmids.

alone or in combination with the Arnt plus AhR plasmids results in a 4- to 5-fold increase in basal CAT activity and a comparable increase in cells treated with 10 nM TCDD. These data contrast to previous transient transfection studies with MDA-MB-231 cells in which transient expression of ER restored Ah-responsiveness using the pRNH11c plasmid but did not affect basal CAT activity [3]. Previous studies with MDA-MB-231 cells cotransfected with pRNH11c plus hER, HE15 or HE19 showed that Ah-responsiveness was restored by expression of either the full length or both truncated ERs [3]. The results summarized in Table 1 show that in Hs578T cells, expression of C-terminal-deleted ER (HE15) resulted in increased basal CAT activity but not restoration of Ah-responsiveness. In contrast, expression of the N-terminal deleted ER (HE19) resulted in a > 47-fold loss of basal activity; however, treatment of these transiently-transfected cells with TCDD caused a 23-fold increase in CAT activity. These results suggest that in Hs578T cells, the various domains of the ER play a differential role in restoration of Ah-responsiveness.

Previous studies have reported higher basal or constitutive expression of CYP1A1 in some breast tumors and this elevated response may be useful as a negative prognostic indicator for breast cancer [11,12]. The results observed in this study with Hs578T cells demonstrate that expression of the full length or C-terminal deleted ER significantly increases constitutive CYP1A1 activity. It has recently been reported that exon 5 deletion variant ER (Δ5ER) mRNA is overexpressed in some tumors and the resulting protein contains TAF-1 but lacks TAF-2 and the ligand-binding domain of the ER [13-15]. These observation are consistent with the enhancement of basal CYP1A1-dependent activity in Hs578T cells by HE15 which is functionally similar to Δ5ER and suggests that future studies on the linkage between expression of Δ5ER and high basal CYP1A1 activity in breast tumors is warranted and may be a useful prognostic indicator for some tumor types. (Supported by the National Institutes of Health, ES03843.)

4. References

1. Vickers, P. J., Dufresne, M. J., and Cowan, K. H. (1989) Relation between cytochrome P4501A1 expression and estrogen receptor content of human breast cancer cells. *Mol. Endocrinol.* 3:157-164.
2. Thomsen, J. S., Nissen, L., Stacey, S. N., Hines, R. N., and Autrup, H. (1991) Differences in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-inducible CYP1A1 expression in human breast carcinoma cell lines involve altered transacting factors. *Eur. J. Biochem.* 197:577-582.
3. Thomsen, J. S., Wang, X., Hines, R. N., and Safe, S. (1994) Restoration of Ah responsiveness in MDA-MB-231 human breast cancer cells by transient expression of the estrogen receptor. *Carcinogenesis* 15:933-937.
4. Pasanen, M., Stacey, S., Lykkesfeldt, A., Briand, P., Hines, R., and Autrup, H. (1988) Induction of cytochrome P-4501A1 gene expression in human breast tumor cells. *Chem. -Biol. Interact.* 66:223-232.
5. Harris, M., Piskorska-Pliszczynska, J., Zacharewski, T., Romkes, M., and Safe, S. (1989) Structure-dependent induction of aryl hydrocarbon hydroxylase in human breast cancer cell lines and characterization of the Ah receptor. *Cancer Res.* 49:4531-4535.
6. Ivy, S. P., Tulpule, A., Fairchild, C. R., Averbuch, S. D., Myers, C. E., Nebert, D. W., Baird, W. M., and Cowan, K. H. (1988) Altered regulation of P-4501AI expression in a multidrug-resistant MCF-7 human breast cancer cell line. *J. Biol. Chem.* 263:19119-19125.
7. Arellano, L. O., Wang, X., and Safe, S. (1993) Effects of cycloheximide on the induction of *CYP1A1* gene expression by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in three human breast cancer cell lines. *Carcinogenesis* 14:219-222.
8. Hines, R. N., Mathis, J. M., and Jacob, C. S. (1988) Identification of multiple regulatory elements on the human cytochrome P4501A1 gene. *Carcinogenesis* 9:1599-1605.
9. Burbach, K. M., Poland, A. B., and Bradfield, C. A. (1992) Cloning of the Ah-receptor cDNA reveals a distinctive ligand-activated transcription factor. *Proc. Natl. Acad. Sci. USA* 89:8185-8189.
10. 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.
11. Pyykkö, K., Tuimala, R., Aalto, L., and Perkiö, T. (1991) Is aryl hydrocarbon hydroxylase activity a new prognostic indicator for breast cancer. *Br. J. Cancer* 63:596-600.
12. Murray, G. I., Foster, C. O., Barnes, T. S., Weaver, R. J., Ewen, S. W. B., Melvin, W. T., and Burke, M. D. (1991) Expression of cytochrome P450IA in breast cancer. *Brit. J. Cancer* 63:1021-1023.
13. Villa, E., Camellini, L., Dugani, A., Zucchi, F., Grottola, A., Merighi, A., Buttafoco, P., Losi, L., and Manenti, F. (1995) Variant estrogen receptor messenger RNA species detected in human primary hepatocellular carcinoma. *Cancer Res.* 55:498-500.
14. Fuqua, S. A. W., Allred, D. C., and Auchus, R. J. (1993) Expression of estrogen receptor variants. *J. Cell. Biochem.* 17G:194-197.
15. Daffada, A. A. I., Johnston, S. R. D., Smith, I. E., Detre, S., King, N., and Dowsett, M. (1995) Exon 5 deletion variant estrogen receptor messenger RNA expression in relation to tamoxifen resistance and progesterone receptor/pS2 status in human breast cancer. *Cancer Res.* 55:288-293.