Effects of Estrogen Receptor Expression on Ah-responsiveness of Adriamycin-Resistant MCF-7 Human Breast Cancer Cells

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Abstract

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) does not induce CYP1A1 gene expression in adriamycin-resistant MCF-7 (MCF-7^{Adr}) cells even though the aryl hydrocarbon receptor (AhR) and AhR nuclear translocator (Arnt) are expressed in these cells. The effects of wild-type human ER (hER) and various ER variants including HE19, HE15 and HE11 which express activation function 2 (AF-2), AF-1 and AF-1/AF-2 (minus the DNA-binding domain) respectively on restoring Ah-responsiveness were investigated in MCF-7^{Adr} using the plasmid pRNH11c which contains the -1142 to +2403 region of the human CYP1A1 gene promoter. In MCF-7^{Adr} cells transiently transfected with pRNH11c and the hER, HE15, HE19, HE11, Arnt or AhR expression plasmids, TCDD induced reporter gene activity only in cells expressing hER or HE15.

Introduction

MCF-7 human breast cancer cell lines have been extensively used as a model for understanding regulation of growth and gene expression. A functional estrogen receptor (ER) is expressed in MCF-7 cells; studies in this laboratory have also characterized the aryl hydrocarbon receptor (AhR) in these cells and have reported AhR-mediated inhibition of 17β-estradiol (E2)induced cell proliferation and gene expression[1-3]. The antiestrogenic activity of relatively non-toxic AhR-based compounds is currently being investigated in this laboratory for their potential use in the clinical treatment of breast cancer.

Most Ah-responsive breast cancer cell lines also express a functional ER and studies in this laboratory have reported that restoration of Ah-responsiveness in ER-negative MDA-MB-231 and Hs578T breast cancer cells by transfecting wild-type or variant human ER (hER) expression plasmids[4, 5]. The interaction between the ER and AhR has been further investigated in ER-negative adriamycin-resistant MCF-7 (MCF-7^{Adr}) cells which have been extensively utilized as a model for studying drug-resistance. Previous studies have been shown that although the AhR is expressed in MCF-7^{Adr} cells , treatment with AhR agonists such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) does not induce CYP1A1 gene expression[6]. This study reports the effects of ER on restoration of Ah-responsiveness in MCF-7^{Adr} cells using an Ah-responsive construct, pRNH11c which contains the -1142 to +2434 region of the human CYP1A1 gene promoter linked to a bacterial chloramphenicol acetyltransferase (CAT) reporter gene[7].

Experimental Methods

Chemicals, Biochemicals and Plasmids: Acetyl CoA were purchased from Sigma (St. Louis, MO). [^{14}C]Chloramphenicol (58.4 mCi/mmol) was obtained from New England Nuclear (Boston, MA). TCDD (>99% pure) was prepared in this laboratory. An hER expression plasmid kindly provided by Dr. Ming Jer Tsai, Baylor College of Medicine (Houston, TX). Deletion mutants of hER expression plasmids (deletions of amino acids of HE11, HE15 and HE19 were 185 to 251, 282 to 595 and 1 to 178, respectively) were kindly provided by Dr. Pierre Chambon. The plasmid pRNH11c (or 11c) 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[7]. Arnt and AhR cDNAs were kindly provided Drs Bradfield and Hankinson[8,9] and constructed into pcDNA1 and pcDNA3 vectors respectively. pcDNA1 and pcDNA3 were purchased from Promega (Madison, WI).

Cell Culture Maintenance and Growth: MCF-7/Adr^{R} cells were kindly provided by Dr. D. Kochevar (Texas A&M University, College Station, TX) and maintained in RPMI medium supplemented with 10% fetal bovine serum (FBS) plus 10 ml antibiotic/antimycotic solution at 37 °C.

Transient Transfection Assay: Cells were seeded in 100-mm Petri dishes and grown until 70% confluence; 5 µg of each plasmid and 20 µg polybrene/ml were used for the assays. After incubation for 6 hr, cells were shocked using 25% DMSO for 4 min followed by treatment with DMSO (0.1% total volume) or TCDD (10 nM) in DMSO for 40 hr in RPMI medium supplemented with 10 % fetal bovine serum. Cells were then washed with phosphate buffered saline (PBS) and scraped from the plates. Cell lysates were prepared in 0.18 ml of 0.25 M 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. The protein concentrations were determined using BSA as a standard. Following TLC, acetylated products were visualized and acetylated band intensities were quantitated using a Betascope 603 Blot analyzer. CAT activity in various treatment groups is expressed relative to that observed in cells transfrected with pRNH11c plus pcDNA3 and treated with DMSO.

Results and Discussion

The results in Figures 1 and 2 summarize the induction of CAT activity in MCF-7^{Adr} cells treated with DMSO (D) or 10 nM TCDD (T) and transiently cotransfected with pRNH11c and pcDNA3 (control vector), hER, Arnt, AhR, HE11, HE15 or HE19 expression plasmids. In cells transiently transfected with pcDNA3, TCDD did not significantly induce CAT activity. In MCF-7^{Adr} cells cotransfected with pRNH11c plus Arnt, AhR or AhR+Arnt (combined) (data not shown) expression plasmids, CAT activity was not induced by TCDD. Interestingly, expression of the AhR increased basal expression of CAT activity whereas Arnt decreased this response. A comparable suppression of basal CAT activity was also observed in cells cotransfected with mutant ER expression plasmids HE11 or HE19 which contain deletion of either the DNA binding or activation function (AF1) domains of the hER respectively. The results show that Ah-responsiveness is restored by wild-type ER or HE15 which expresses the N-terminal AF-1 domain of the ER. These results show the importance of cellular context on the restoration of Ah-responsiveness by the wild-type and variant ER expression plasmids; for example, inducibility of CAT activity by TCDD is observed after cotransfecting hER, HE15 or HE19 (MDA-MB-231)[4] , only HE19 (Hs578T)[5] or hER and HE15 (MCF-7^{Adr}) respectively. Current studies are focused on understanding ER-mediated effects on the AhR and its function in ER-negative breast cancer cells.

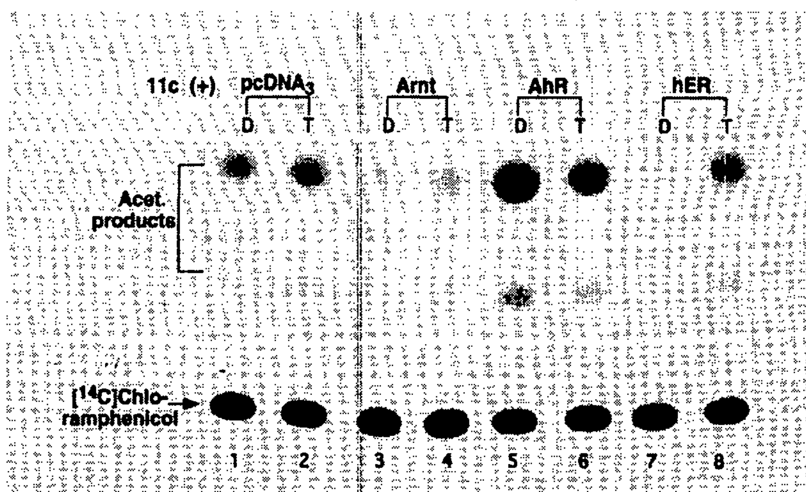

Figure 1. Effects of Arnt, AhR and wild-type hER on restoration of Ah-responsiveness in MCF-7/Adr^R human breast cancer cells. The cells were transiently transfected with 5 μg of pRNH11c (11c) and co-transfected with 5 μg of other expression plasmids for each different experiment. Lanes 1, 3, 5 and 7 were treated with DMSO (D), whereas lanes 2, 4, 6 and 8 were treated with 10 nM TCDD (T) for 40 h. CAT assay was carried out as described in the "Materials and Methods".

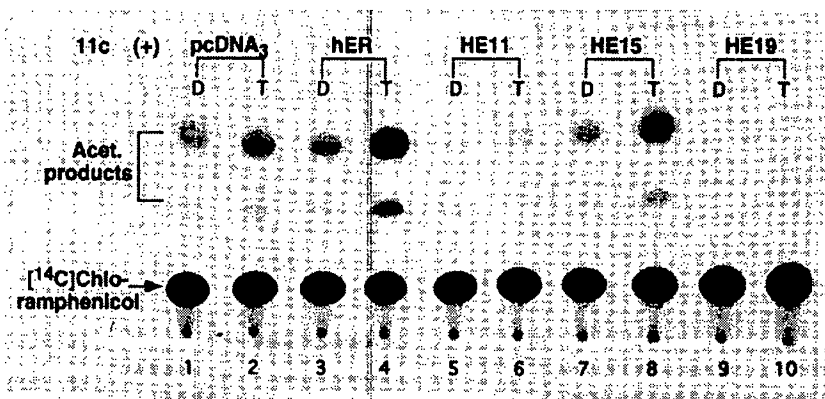

Figure 2. Effects of wild-type and mutant hERs on restoration of Ah-responsiveness in MCF-7/Adr^R human breast cancer cells. The cells were transiently transfected with 5 μg of pRNH11c (11c) and co-transfected with 5 μg of other expression plasmids for each different experiment. Lanes 1, 3, 5, 7 and 9 were treated with DMSO (D), whereas lanes 2, 4, 6, 8 and 10 were treated with 10 nM TCDD (T) for 40 h. CAT assay was carried out as described in the "Materials and Methods".

Acknowledgments

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