RESTORATION OF Ah-RESPONSIVENESS IN MDA-MB-231 HUMAN BREAST CANCER CELLS BY THE ESTROGEN RECEPTOR

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ABSTRACT
Treatment of the MDA-MB-231 human breast carcinoma cell line with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) resulted in accumulation of the liganded Ah receptor complex in the nucleus, but no induction of the *CYP1A1* gene was observed. Transient transfection of MDA-MB-231 cells with the plasmid pRNH11c, containing the intact human CYP1A1 regulatory region from -1142 to +2434 fused to the reporter gene, *chloramphenicol acetyl transferase* (CAT) showed no TCDD-induced CAT-expression, indicating that the lack of *CYP1A1* induction is due to altered transcription factors rather than mutated dioxin responsive elements (DRE's). MDA-MB-231 cells do not express a functional estrogen receptor (ER), a phenotype shared by other human breast cancer cell lines. To elucidate, whether a functional ER is required for Ah-responsiveness in MDA-MB-231 cells, a vector containing the human ER cDNA, ΔhER, was transfected into the cells. The basal CAT-activity did not change in the presence of the ΔhER-plasmid. However, in cells treated with 10 nM TCDD, a significant increase in CAT-activity was observed. Increasing the amount of ΔhER-plasmid transfected into the cells further increased CAT-activity. Transfecting the cells with the more simple construct, pMCAT 5.12, containing one DRE in front of the MMTV promoter, mimicked the results obtained with pRNH11c, indicating that the restoration of TCDD-responsiveness by ER is mediated via the Ah receptor-DRE interaction. To test, if the restoration of the function of the Ah receptor was due to overexpression of an unspecific nuclear factor, cells were transfected with a plasmid encoding the c-Jun protein. No TCDD-induced CAT expression was observed. Cells cotransfected with plasmids encoding for c-Jun and ER resulted in decreased TCDD-induced CAT-activity compared to cells transfected with ΔhER alone. Since c-Jun is known to downregulate the amount of ER, the results support he hypothesis that the concentration of ER is a determining factor in restoration of the function of the Ah receptor.

INTRODUCTION

Induction of *CYP1A1* gene expresion by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is mediated through the nuclear Ah receptor, which binds to genomic dioxin responsive elements (DRE's) present in several copies upstream of the *CYP1A1* gene. However, in the MDA-MB-231 human breast carcinoma cell line, no induction of *CYP1A1* was observed despite the

+2434. Only minimal TCDD-induced CAT expression was observed (Figure A, lanes1-2). Cells were then cotransfected with the reporter plasmid, pRNH11c and a plasmid containing the human ER cDNA, ΔhER. As illustrated in figure A, lane 4, a significant increase in TCDD-induced CAT expression was observed, whereas no response was observed in control cells (lane 3). Increasing the amount of ER transfected into the cells further increased the TCDD-induced CAT-activity (lane 5) indicating a positive correlation between the amount of transfected ER and TCDD-responsiveness.

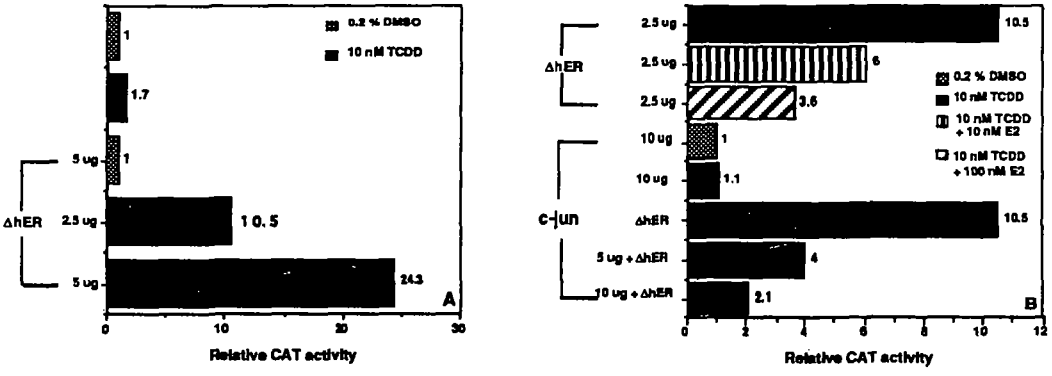

Figure A: MDA-MB-231 cells were transfected with the reporter plasmid, pRNH11x (5 μg) ±ΔhER, the vector encoding human ER. Cells were treated for 48 hr before harvest with either 10 nM TCDD or vehicle alone (0.2 % DMSO). Figure B: MDA-MB-231 cells were transfected with 5 μg pRNH11c. Whenever transfected with ΔhER, 2.5 μg was used. Lanes 4-5 and 7-8 represent activities from cells cotransfected with c-jun-plasmid as indicated. Treatments were either vehicle alone (0.2 % DMSO) (lane 4), 10 nM TCDD (lanes 1, 5-8) or cotreatment of 10 nM TCDD and E_2 (10 nM in lane 2) (100 nM in lane 3).

Replacing the CAT marker plasmid, pRNH11c, with the more simple construct, pMCAT 5.12, containing a single DRE in front of the MMTV promoter, mimicked the results obtained with pRNH11c (data not shown), indicating that the restoration of TCDD-responsiveness by ER is mediated via the Ah receptor-DRE interaction.

Cotreatment of the cells with TCDD and estradiol (E_2) decreased the CAT induction in a E_2-dependent manner (figure B, lanes 2-3). E_2 has been shown to downregulates the amount of ER posttransriptionally in MDA-MB-231 cells stably transfected with ER [12], indicating that it is the presence of ER that plays a pivotal role in the restoration of the function of the Ah receptor.

In order to investigate the specificity of interaction between ER and the Ah receptor, the cells were transfected with a vector encoding an unrelated protein, c-Jun. No TCDD-induced CAT expression was observed (figure B lane 5) ruling out the possibility that restoration of the Ah receptor function is due to overexpression of an unspecific nuclear factor. TCDD-treated cells cotransfected with the plasmid c-Jun and ΔhER expressed less CAT-activity than cells transfected with ΔhER alone (figure B lanes 7-8); c-Jun has been reported to downregulate

presence of Ah receptors[1]. In TCDD-treated cells, gel mobility shift analysis of nuclear extracts from the cells incubated with a synthetic DRE probe gave a retarded band[1], thus showing that the Ah receptor complex recognizes and bind to a DRE in a TCDD-dependent manner. Transient transfection of MDA-MB-231 cells with the plasmid. pRNH11c, containing the intact human *CYP1A1* regulatory region from -1142 to +2434 fused to the reporter gene, *chloramphenicol acetyl transferase* (CAT)[2] showed no TCDD-induced CAT-expression, indicating that the lack of *CYP1A1* induction was due to an impaired Ah receptor rather than mutated DRE's, since this construct contains intact DRE's. MDA-MB-231 cells do not express a functional estrogen receptor (ER), and thus resemble other ER-negative human breast carcinoma cell lines[3,4]. In this study, the effect of a functional ER on the restoration of Ah receptor-mediated responses in the MDA-MB-231 cell line will be investigated.

MATERIALS AND METHODS

Cell Growth: The human adenocarcinoma cell line, MDA-MB-231, was obtained from the American Type Culture Collection (Rockville, MD). Cells were grown in DME-F12 medium without phenol red and supplemented with 5% fetal bovine serum plus 10 ml antibiotic-antimycotic solution (Sigma) and 25 mM sodium bicarbonate.

Transient CAT transfection Assay: The plasmid, pRNH11c, contains the untranslated human CYP1A1 sequence from the TaqI site at -1142 to the BclI site at +2434 fused to the CAT reporter gene CAT[2]. The plasmid, ΔhER, contains the human estrogen receptor cDNA, in which the short open reading frame near the 5'end of the sequences upstream of the TthI site has been deleted[5]. The plasmid, pMCAT 5.12 containing a mouse DRE fused to the MMTV promoter driving the CAT gene was a gift from Dr. J.P. Whitlock Jr., Stanford University. The c-Jun expression vector is under RSV control [6].
Cells were seeded on 60 mm petri dishes and grown until 75% confluent. The transfection was carried out essentially as described[7,8] using 5 μg plasmid DNA and 20 μg/ml polybrene. Six hr later cells were shocked using 35 % DMSO for 4 min. Treatment of the cells with TCDD (10 nM), E2 (10 nM, 0.1 μM), and/or DMSO was performed 18 hr after DMSO shock. After 48 hr, the cells were washed 3 times with phosphate-buffered saline and scraped from the plates. Cell lysates were prepared in 0.2 ml 0.25 M Tris/HCl pH 7.8 by 3 freeze/thaw cycles, and sonication (4 min) in an ultrasonic bath (Branson). Cell lysates were incubated at 56°C for 10 min to remove endogenous deacetylase activity[9]. CAT activity was determined as described[10], using 0.2 μCi d-threo- [dichloroacetyl-1-14C] chloramphenicol (54 Ci/mol, Amersham) and 4 mM acetyl-CoA as substrates. The protein concentration was determined using bovine serum albumin as a standard[11]. Following thin-layer chromatography (TLC), the regions of the TLC plate containing acetylated products were visualized by autoradiography and quantitated by using a Betascope 603 Blot Analyzer.

RESULTS AND DISCUSSION

In order to elucidate whether the lack of TCDD-responsiveness in the MDA-MB-231 cell line was due to altered cis- or trans-acting factors, the cell line was transfected with the CAT-plasmid pRNH11c, containing the intact human *CYP1A1* regulatory region from -1142 to

the concentration of ER[6].Whether the restoration of the function of the Ah receptor is due to a direct interaction between ER and the Ah receptor or whether it is a squelching effect - an outtitration of a negative factor inhibiting the Ah receptor from activating transcription, is currently under investigation.
(Supported by the National Institutes of Health ESO3843)

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