Regulation of Rat Liver Epithelial Cell Growth and Epidermal Growth Factor Receptor by TCDD

C. H. Sewall†,‡, G. W. Lucier†, A. M. Tritscher†, and G. C. Clark†

†National Institute of Environmental Health Sciences, P.O. Box 12233, MD D4-04, RTP, NC 27709; and ‡University of North Carolina, Curriculum in Toxicology, Chapel Hill, NC

1. Introduction

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a potent tumor promoter and a powerful dysregulator of epithelial cell growth homeostasis. It has previously been shown that TCDD causes a decrease in epidermal growth factor receptor (EGFR) levels in a chronic rat liver tumor promotion study as well as in *in vitro* studies with human keratinocytes. This has led to the hypothesis that alterations in EGFR and related signal transduction pathways may play a role in TCDD's effects on cell growth and differentiation. The objective of the present study was to establish an *in vitro* model for investigating the mechanism of TCDD induced changes in EGFR. The WB liver epithelial cell line[1),2)] was selected because: 1) it is derived from rat liver, a target organ of TCDD toxicity, 2) it is continuous yet nontransformed, and 3) it has an intact EGFR sytem comparable to normal hepatocytes.

2. Background

A decade ago Madhukar and collaborators[3)] noted that many of the effects of TCDD were similar to that induced by exogenous EGF, and based on the finding that TCDD reduced EGF binding in hepatic plasma membrane of several rodents, hypothesized that the action of TCDD on the EGF receptor may contribute to some of TCDD's alterations of epithelial tissues. Additional studies have further characterized TCDD dependent changes of EGFR in liver,[4),5),6)] uterine,[7)] and embryonic ureteric epithelium and palatal medial epithelial cell.[8)]. Decreased EGFR binding capacity has also been demonstrated in cultured human keratinocytes exposed to TCDD.[9),10)] Based on these studies it is concluded that TCDD does not bind to the EGFR and that the changes in EGFR likely result from an Ah receptor mediated pathway. Although it has been proposed that alterations in the EGFR by TCDD may contribute to changes in

cell growth, there is little direct evidence that describes the molecular events that lead to the changes in EGFR.

3. Methods

Rat liver epithelial cells, provided by Dr. Grisham (University of North Carolina, Chapel Hill), were grown in Richter's media with 10 % FCS. All experiments were performed on confluent cells at passages 22-30. CYP 1A1 activity was measured by the ethoxyresorufin-o-deethylase enzyme assay. Cytosolic fractions were isolated and the presence of the Ah receptor was determined by photoaffinity labeling and immunoblot analysis. Cell surface EGF receptor was quantified by equilibrium binding with radiolabeled EGF while total cellular EGF receptor was assessed by immunoblot detection. The effect of TCDD on liver epithelial cell growth was determined by electronic cell counting as well incorporation of tritiated thymidine into DNA. The cells were metabolically labeled with [^{35}S]methionine using pulse-chase techniques followed by the immunoprecipitation of the EGFR and detection by fluorography to determine the rate of EGFR synthesis and turnover. The activation status and function of the EGFR was determined by immunoblot analysis of phosphotyrosine residues as well as binding to growth factor receptor-bound protein-2 (GRB2). Steady-state EGFR mRNA levels were investigated using Northern blot analysis.

4. Results

We report here that the WB cell, a previously characterized nontransformed rat liver epithelial cell line, expresses high levels of Ah receptor and is responsive to TCDD as determined by induction of CYP1A1, a target gene transcriptionally regulated by TCDD-Ah receptor complex. The dose-response for CYP1A1 induction was similar to that for increases in cell proliferation as measured by increased DNA synthesis and cell number (ED_{50}=0.05 nM TCDD). The increase in DNA synthesis coincided with a decrease in total EGFR levels. The mechanism of EGFR decrease was investigated by assessing: 1) the rate of EGFR synthesis and degradation by pulse-chase methods, 2) changes in EGFR mRNA by Northern analysis (see Figure 1), and 3) alterations in EGFR activation status as determined by phosphotyrosine residues and binding to GRB2. The results suggest that the mechanism of action for TCDD induced decreases in EGFR is transcriptional as evidenced by decreases in EGFR mRNA and rate of EGFR synthesis, with no change in EGFR rate of degradation or activation status.

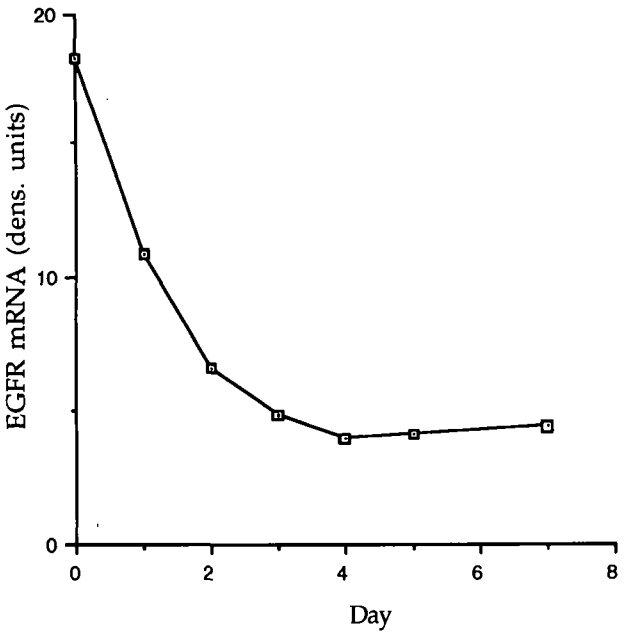

Figure 1. Time course of EGF receptor mRNA decrease. Total RNA was isolated from control cells and cells treated with TCDD (0.3 nM) at the indicated times prior to lysis. After electrophoresis in 0.8% agarose gel, the RNA was analyzed by Northern analysis. The 9.6 kb transcript encoding the full-length EGFR was quantified by scanning laser densitometry.

5. Conclusions

In this study we have shown that TCDD decreased the EGFR protein in rat liver epithelial cells. Based on metabolic labeling and Northern analysis a possible mechanism for the decrease is transcriptional repression. However additional studies are necessary to address the issue of mRNA stability as well as investigate possible interactions at the level of the EGFR promoter region since several hormones and transcription factors have been shown to result in transcription suppression of the EGFR gene. In conclusion this study has established that the WB cell is a sensitive and responsive cell line that is a useful *in vitro* model for understanding the *in vivo* observations of TCDD's decrease in hepatic EGFR.

5. References

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