Epidermal Growth Factor Receptor Kinase Inhibitors Synergize with TCDD to Induce CYP1A1/1A2 in Human Breast Epithelial MCF10A Cells

Aby Joiakim,1 Patricia A. Mathieu, Catherine Shelpe,2 Julie Boerner, and John J. Reiners, Jr.

Institute of Environmental Health Sciences, Wayne State University, Detroit, Michigan (A.J., P.A.M., J.J.R.); Department of Pharmacology (C.S., J.J.R.) and Department of Oncology (J.B.), Wayne State University School of Medicine, Karmanos Cancer Institute, Detroit, Michigan

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ABSTRACT

CYP1A1 and CYP1A2 are transcriptionally activated in the human normal breast epithelial cell line MCF10A following exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Shifting MCF10A cultures to medium deficient in serum and epidermal growth factor (EGF) caused rapid reductions in the activated (i.e., phosphorylated) forms of extracellular regulated kinases (ERKs) and the epidermal growth factor receptor (EGFR). Shifting to serum/EGF-deficient medium also enhanced TCDD-mediated induction of cytochrome P450 (CYP1A1). Treatment of cells cultured in complete medium with the EGFR inhibitors gefitinib, AG1478, and CI-1033 resulted in concentration-dependent reductions of active EGFR and ERKs, and increased CYP1A1 mRNA content ∼3- to 18-fold above basal level. EGFR inhibitors synergized with TCDD and resulted in transient CYP1A1 and CYP1A2 mRNA accumulations ∼8-fold greater (maximum at 5 hours) than that achieved with only TCDD.

Introduction

The aryl hydrocarbon receptor (AhR) is a ubiquitously expressed, ligand-activated cytosolic receptor. Its activating ligands include several natural products as well as environmental pollutants such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), polychlorinated biphenyls, and some polycyclic aromatic hydrocarbons (Denison et al., 2011). Upon ligand binding the activated AhR translocates to the nucleus, whereupon it complexes with the arylhydrocarbon receptor nuclear translocator protein, binds to xenobiotic response elements (XREs) in the promoters of AhR responsive genes, and recruits coactivators or corepressor proteins that modulate the expression of target genes (Denison et al., 2011). Although early studies focused on AhR-mediated transcriptional activation of a subset of cytochrome P450s (CYPs), such as CYP1A1 and CYP1A2, a large number of genes have been subsequently characterized whose transcription is regulated by the AhR. Although the effects of the AhR are generally attributed to its direct effects on the transcription of target genes, it has increasingly become clear that the liganded-AhR can influence a variety of processes by nontranscriptional mechanisms. The epidermal growth factor receptor (EGFR) is one such example.

The EGFR/ErbB1 is the founding member of the ErbB family. The family consists of EGFR, ErbB2/HER2, ErbB3, and ErbB4 receptors (Yarden and Sliwkowski, 2001). The EGFR, ErbB3 and ErbB4 bind a diverse array of activating ligands. Whereas ligand-bound EGFR and ErbB4 transmit their signaling as homodimers or as a heterodimer with another member of the ErbB family, ErbB2 and ErbB3 to be functionally active must form heterodimers with other ErbB members (Yarden and Sliwkowski, 2001, Nickerson et al., 2011). Receptor and ligand expression are tissue-specific, in many cases developmentally regulated, and can be influenced by exogenous factors as well as the physiologic/pathologic...
Numerous studies indicate that AhR ligands can influence EGFR signaling. Initially, it was noted that polycyclic aromatic hydrocarbon pretreatment of cultured cells reduced the binding of exogenously added epidermal growth factor (EGF) to the plasma membrane (Ivanovic and Weinstein, 1982; Kärenlampi et al., 1983). Thereafter, studies appeared documenting the effects of gestational TCDD exposure on embryonic EGF levels in and during development of salivary gland (Kiuikkonen et al., 2006), palate (Abbott and Birnbaum, 1990), and urogenital bud (Bryant et al., 1997). Subsequent studies employing in vivo and cell culture models demonstrated that TCDD could activate the EGFR via AhR-dependent processes [for review see Haarmann-Stemmann et al. (2009)]. These processes include both a transcriptional mechanism in which TCDD stimulates the transcription of EGF-activating ligands (Patel et al., 2006), as well as a transcriptionally independent process in which Src kinase is released from cytoplasmic AhR-chaperone-Src complexes following the binding of TCDD. Subsequent phosphorylation of the EGFR by Src results in ligand-independent activation of the EGFR (Biscardi et al., 1999). Additional AhR-dependent, transcriptional-independent mechanisms for TCDD-dependent activation of the EGFR have been described (Haarmann-Stemmann et al., 2009).

Whereas AhR-mediated modulation of EGFR activation and signaling is fairly well documented, relatively little is published on the converse. What is known is derived from analyses of TCDD effects on developmental processes in transforming growth factor-α- or EGF-deficient mice (Bryant et al., 2001; Abbott et al., 2003), and the effects of exogenously added EGF on the mRNA and protein contents of AhR-responsive genes (e.g., primarily CYP1A1/1A2) in cultured cells (Bachleda et al., 2009; Sutter et al., 2009). The results of these latter studies suggest that the activated EGFR mutes AhR signaling. In the current study we determined if small-molecule inhibitors of the ErB family modulate basal and TCDD-inducible expression of CYP1A1 and CYP1A2 in the normal human breast epithelial cell line MCF10A, and the human mammary carcinoma MCF7-TETon-EGFR cell line. We chose the former because it expresses both the AhR and EGFR, with the EGFR being activated by exogenously added EGF. We chose the latter because it has a functional AhR and offered the opportunity to regulate the expression of the EGFR (Boerner et al., 2005). Our studies indicate that inhibitors of the EGFR, but not ErB2/HER2, markedly enhance both basal and AhR-mediated accumulation of CYP1A1/1A2 mRNA in MCF10A, but not MCF7-TETon-EGFR, cells. Hence, within the appropriate cellular context, EGFR signaling may function as a rheostat to control AhR signaling.

**Materials and Methods**

**Materials.** TCDD was obtained from Midwest Research Institute (Kansas City, MO). Epidermal growth factor, penicillin/streptomycin solution, horse serum, TRIzol, and trypsin/EDTA were purchased from Invitrogen/ThermoFisher Scientific (Waltham, MA). Dimethylsulfoxide was obtained from SigmaMillipore (St. Louis, MO). Radioimmunoprecipitation assay lysis buffer, HALT Protease Inhibitor Cocktail, and HALT Phosphatase Inhibitor Cocktail were obtained from ThermoFisher Scientific. Cl-1033 and Pd184352 were originally obtained as gifts from Pfizer, and CI-1033 and PD184352 were purchased from Selleckchem (Houston, TX). Gefitinib (Iressa) was originally obtained as a gift from AstraZeneca and subsequently purchased from SigmaMillipore (St. Louis, MO). Epidermal growth factor, penicillin/streptomycin solution, horse serum, TRIzol, and Pd184352, and trypsin/EDTA were purchased from Invitrogen/ThermoFisher Scientific (Waltham, MA). Dimethylsulfoxide was obtained from SigmaMillipore (St. Louis, MO). Radioimmunoprecipitation assay lysis buffer, HALT Protease Inhibitor Cocktail, and HALT Phosphatase Inhibitor Cocktail were obtained from ThermoFisher Scientific. Cl-1033 and Pd184352 were originally obtained as gifts from Pfizer, and CI-1033 and PD184352 were purchased from Selleckchem (Houston, TX). Gefitinib (Iressa) was originally obtained as a gift from AstraZeneca and subsequently purchased from SigmaMillipore (St. Louis, MO). Dimethylsulfoxide was obtained from SigmaMillipore (St. Louis, MO). Radioimmunoprecipitation assay lysis buffer, HALT Protease Inhibitor Cocktail, and HALT Phosphatase Inhibitor Cocktail were obtained from ThermoFisher Scientific. Cl-1033 and Pd184352 were originally obtained as gifts from Pfizer, and CI-1033 and PD184352 were purchased from Selleckchem (Houston, TX). Gefitinib (Iressa) was originally obtained as a gift from AstraZeneca and subsequently purchased from SigmaMillipore (St. Louis, MO). Dimethylsulfoxide was obtained from SigmaMillipore (St. Louis, MO). Radioimmunoprecipitation assay lysis buffer, HALT Protease Inhibitor Cocktail, and HALT Phosphatase Inhibitor Cocktail were obtained from ThermoFisher Scientific. Cl-1033 and Pd184352 were originally obtained as gifts from Pfizer, and CI-1033 and PD184352 were purchased from Selleckchem (Houston, TX). Gefitinib (Iressa) was originally obtained as a gift from AstraZeneca and subsequently purchased from SigmaMillipore (St. Louis, MO). Dimethylsulfoxide was obtained from SigmaMillipore (St. Louis, MO). Radioimmunoprecipitation assay lysis buffer, HALT Protease Inhibitor Cocktail, and HALT Phosphatase Inhibitor Cocktail were obtained from ThermoFisher Scientific. Cl-1033 and Pd184352 were originally obtained as gifts from Pfizer, and CI-1033 and PD184352 were purchased from Selleckchem (Houston, TX). Gefitinib (Iressa) was originally obtained as a gift from AstraZeneca and subsequently purchased from SigmaMillipore (St. Louis, MO). Dimethylsulfoxide was obtained from SigmaMillipore (St. Louis, MO). Radioimmunoprecipitation assay lysis buffer, HALT Protease Inhibitor Cocktail, and HALT Phosphatase Inhibitor Cocktail were obtained from ThermoFisher Scientific. Cl-1033 and Pd184352 were originally obtained as gifts from Pfizer, and CI-1033 and PD184352 were purchased from Selleckchem (Houston, TX). Gefitinib (Iressa) was originally obtained as a gift from AstraZeneca and subsequently purchased from SigmaMillipore (St. Louis, MO). Dimethylsulfoxide was obtained from SigmaMillipore (St. Louis, MO). Radioimmunoprecipitation assay lysis buffer, HALT Prot...
(i.e., gefitinib and AG1478; Levitzki and Gazit, 1995; Arora and Scholar, 2005), for ErbB2/HER2 (i.e., AG825; Levitzki and Gazit, 1995), or for all members of the ErbB family (i.e., CI-1033; Arora and Scholar, 2005). As a screen for EGFR activation we monitored EGFR phosphorylation at tyrosine 1068, one of the three major autophosphorylation sites on the EGFR, and a nexus for recruitment of adaptor molecules that link the receptor to downstream signaling pathways such as Ras.

The EGFR inhibitors gefitinib and AG1478 (Fig. 1A), and the panning ErbB family inhibitor CI-1033 (Fig. 1B), caused concentration-dependent reductions in phosphorylated (i.e., activated) EGFR and extracellular regulated kinase (ERK) in MCF10A cultures. With each inhibitor the losses of phosphorylated EGFR and phosphorylated ERK paralleled one another over the range of tested concentrations. Interestingly, whereas loss of phosphorylated EGFR was paralleled by a reciprocal accumulation of total EGFR in gefitinib- or AG1478-treated cultures (Fig. 1A), loss of phosphorylated EGFR was paralleled by the loss of total EGFR in CI-1033-treated cultures (Fig. 1B).

**EGFR Inhibitor Enhancement of CYP1A1 Induction.** Preliminary studies indicated that pretreatment with gefitinib dramatically enhanced the accumulation of CYP1A1 mRNA in TCDD-treated cultures. The extent to which this occurred necessitated the use of exceptionally short exposures (in Northern blot analyses) to avoid underestimating the magnitude of the enhancement (data not shown).

**Fig. 1.** EGFR kinase inhibitors modulate EGFR and ERK phosphorylation. Cultures of MCF10A cells were left untreated, or treated with dimethyl sulfoxide or 10 nM TCDD ± varied concentrations of (A) gefitinib or AG1478, or (B) CI-1033. The EGFR inhibitors were added 30 minutes prior to TCDD. Cultures were harvested for Western blot analyses 1 hour after dimethyl sulfoxide or TCDD treatment. Each lane contained 25 μg of protein. Similar results were obtained in two additional independent experiments.

**Fig. 2.** EGFR kinase inhibitor enhancement of TCDD-mediated induction of CYP1A1. MCF10A cultures were left untreated (NT), or treated with 10 nM TCDD, varied concentrations of gefitinib (A,D) or AG1478 (B,D) or CI-1033 (C), or TCDD + gefitinib/AG1478/CI-1033 (A-D). EGFR inhibitors were added 30 minutes [15 minutes for studies in (D)] prior to TCDD addition. Cultures were harvested 5 hours after TCDD addition for mRNA isolation for Northern blot (A–C) or quantitative real-time PCR analyses of CYP1A1 mRNA. Results similar to those reported in panels (A)–(C) were obtained in two additional independent experiments. Data in (D) represent means ± S.D. (N = 3 independent samples).

*Greater than NT or dimethyl sulfoxide–treated groups (P < 0.05). †Greater than NT, dimethyl sulfoxide–, or TCDD-treated groups (P < 0.05). The vertical line separating lanes in (A) represent the point at which the scan was cut and the gefitinib treatments were flipped so as to be consistent with the high dose to low dose orientation used in the other panels.
EGFR phosphorylation (compare Fig. 1A with Fig. 2 A, B, and D, and compare Fig. 1B with Fig. 2C).

Real time PCR analyses indicated that gefitinib and AG1478 alone, if used at concentrations sufficient to suppress EGFR activation, caused small but statistically significant accumulations of CYP1A1 mRNA (~3- to 18-fold above basal levels, Fig. 2D). CYP1A1 mRNA content, after combined TCDD and EGFR inhibitor treatment, greatly exceeded the theoretical mRNA content calculated by summing the effects of the individual agents (Fig. 2D). Hence it appears that TCDD and EGFR inhibitors synergize in their induction of CYP1A1.

Kinetic analyses indicated that CYP1A1 mRNA accumulation reached a steady state within ~3 hours of TCDD treatment, which was maintained for at least an additional 18 hours (Supplemental Fig. 2). Maximum accumulation of CYP1A1 mRNA following combinational TCDD plus gefitinib or AG1478 treatment occurred within 3–5 hours of TCDD addition. Thereafter, CYP1A1 mRNA content decreased until 13 hours post-treatment, at which time it was comparable to CYP1A1 mRNA content measured in cultures treated with only TCDD (Supplemental Fig. 2).

EGFR Inhibitor Effects on CYP1A1 Protein Levels. Although CYP1A1 mRNA contents increased ~60-fold following TCDD exposure, CYP1A1 protein contents were only marginally increased (~1.2- to 1.9-fold) at 6 hours (Fig. 3, A and B) and 11 hours (Fig. 3C) after treatment. The EGFR inhibitors gefitinib and AG1478 also increased CYP1A1 protein contents approximately 1.6- to 2.5-fold during the same time period (Fig. 3). No additive or synergistic effects on CYP1A1 protein content were observed following combinational treatment (Fig. 3). Results similar to those reported in Fig. 3 were obtained in two additional experiments spanning 6–23 hours of TCDD treatment.

**EGFR Inhibitor Enhancement of CYP1A2 Induction.** The activated AhR, in addition to effecting CYP1A1 transcription, also induces CYP1A2 transcription in MCF10A cells (Elliott et al., 2012). Pretreatment of MCF10A cultures with 500 nM gefitinib or AG1478, concentrations sufficient to suppress EGFR phosphorylation, dramatically increased the accumulation of CYP1A2 mRNA in TCDD-treated cultures (Fig. 4). Pretreatment with AG825, a structural analog of AG1478 that inhibits ErbB2, but not the EGFR (Levitzki and Gazit, 1995), had no effect on CYP1A2 induction by TCDD (Fig. 4).

**EGF Modulation of CYP1A1 Induction.** Shifting MCF10A cultures to serum- and EGFR-deficient medium resulted in rapid reductions in total and phosphorylated/activated EGFR, which were paralleled by the loss of phosphorylated/activated ERK (Fig. 5A). Shifting cultures to serum- and EGFR-deficient medium prior to TCDD addition also resulted in CYP1A1 mRNA accumulations comparable to those occurring in cultures maintained in complete medium, and cotreated with TCDD and gefitinib (Fig. 5B) or AG1478 (Fig. 5C). No additional CYP1A1 mRNA accumulation occurred if cultures were shifted to deficient medium prior to cotreatment with TCDD and either gefitinib (Fig. 5B) or AG1478 (Fig. 5C). Collectively, these studies suggest that the effects of the EGFR inhibitors on CYP1A1 mRNA content are mediated by their suppression of EGFR activity.

**Modulation of CYP1A1 Induction by PD184352.** The EGFR activates several signaling pathways, one of which is the Raf-MEK-ERK pathway. Concentration-response analyses indicated a direct relationship between the extent to which EGFR inhibitors synergized with TCDD in the induction of CYP1A1 and reduced activated ERK content. The use of MAPK kinase (MEK) inhibitors PD98059 and U0126 for investigation of AhR activation/signaling is confounded by the possibility that they may be AhR ligands, and function as agonists or antagonists (Reiners, et al., 1998; Andrieux et al., 2004; Chen et al., 2005). PD184352 is structurally unrelated to PD98059 and U0126, and is a potent MEK inhibitor (Frémion and Meloche, 2010). Electrophoretic mobility shift assays demonstrated that concentrations of PD184352 ≤ 50 μM did not transform rat liver AhR into an XRE-binding species, whereas concentrations >1 μM weakly suppressed TCDD-mediated transformation of rat liver AhR into an XRE-binding species (Supplemental Fig. 3). It is important to note that this latter activity occurs at a concentration far greater than that needed to suppress MEK activity in MCF10A cultures (Fig. 6A).

Exposure of MCF10A cultures to ≥25 nM PD184352 eliminated ERK activity in TCDD-treated cultures (Fig. 6A). At lower MEK inhibitor concentrations pERK content appeared to be elevated. This increase was not a consequence of the MEK inhibitor. Rather, TCDD alone significantly elevated pERK content within 2 hours of treatment (Fig. 6B). Other investigators have reported a similar result (Tan et al., 2002; Puga et al., 2009). Concentrations of PD184352 sufficient to...
potentially inhibit ERK phosphorylation also caused a minor reduction in ERGFTyr1068 phosphorylation (Fig. 6B). Concentrations of PD184352 sufficient to inhibit ERK activity had little effect on basal CYP1A1 mRNA content (Fig. 6D). However, when used in combination with TCDD, concentrations of PD184352 sufficient to inhibit ERK dramatically enhanced the accumulation of CYP1A1 mRNA (Fig. 6, C and D). Cotreatment with gefitinib did not further enhance the potentializing effect of PD184352 on CYP1A1 induction by TCDD (Reiners, unpublished data).

**Effects of EGFR and MEK Inhibitors on CYP1A1 Induction in MCF7 Derivatives.** MCF7 cells express very little EGFR (Boerner et al., 2005). The MCF7-TETon-EGFR cell line is an MCF7 derivative that expresses a functional EGFR if cultured in the presence of doxycycline (Boerner et al., 2005). In the absence of doxycycline MCF7-TETon-EGFR cultures expressed activated ERK but no EGFR (Fig. 7A). ERK activities were suppressed by PD184352, but not by gefitinib or CI-1033 treatment (Fig. 7A). Neither PD184352 nor the two EGFR kinase inhibitors influenced the induction of CYP1A1 by TCDD in MCF7-TETon-EGFR cells cultured in the absence of doxycycline (Fig. 7B).

In the presence of doxycycline, MCF7-TETon-EGFR cultures expressed the EGFR, and it was active, on the basis of analyses of Tyr1068 phosphorylation (Fig. 7A). Treatment with either gefitinib or CI-1033 suppressed EGFR phosphorylation, and reduced total EGFR protein, but had no effect on ERK phosphorylation (Fig. 7A). This lack of effect suggests that the activated EGFR is not coupled to the MAPK pathway in MCF7 cells. As expected, PD184352 suppressed ERK phosphorylation (Fig. 7A). None of the inhibitors influenced the induction of CYP1A1 by TCDD in doxycycline-treated MCF7-TETon-EGFR cultures (Fig. 7B).

**Discussion**

The current study indicates that EGFR-mediated signaling modulates the induction of CYP1A1 by TCDD in MCF10A cells. This statement is supported by the findings that the induction of CYP1A1 by TCDD was enhanced either by pretreating cultures with small-molecule EGFR inhibitors or by shifting MCF10A cultures to EGFR-deficient medium prior to TCDD addition. In the case of the EGFR inhibitor studies, concentration-response studies indicated an inverse relationship between EGFR activity and the level to which TCDD could transcriptionally activate CYP1A1. Since EGFR inhibitors also enhanced the induction of CYP1A2 by TCDD, it appears that EGFR-mediated signaling dampens AhR function in MCF10A cells. Notably, because the ErbB2 inhibitor AG825 did not influence the induction of CYP1A2 by TCDD, it is improbable that EGFR/ErbB2 heterodimers mediate the suppressive activity of the EGFR.

The dampening effect of the activated EGFR on ligand-activated AhR signaling has been noted by others. Specifically, Sutter et al. (2009) reported that the addition of EGF to confluent primary human keratinocyte cultures markedly suppressed the induction of CYP1A1 by TCDD, as well as transcription initiated from reporter constructs.
in a series of EGFR wild-type non-small cell lung carcinoma (NSCLC) cell lines treated with gefitinib. The effects of gefitinib were presumably mediated by its interaction with the EGFR, since comparable results did not occur in NSCLC cell lines resistant to gefitinib owing to mutant EGFRs. Microarray analyses of a human glioblastoma cell line treated with camptothecin, an inhibitor of EGFR activation, also revealed an accumulation of mRNAs corresponding to AhR-responsive genes (Konkimalla and Efferth, 2010).

The EGFR serves as a platform and nexus for several signaling pathways, including the Ras/RAF/MEK/ERK pathway. ERK activity appears to be tightly coupled to EGFR activity in MCF10A cells, because pharmacological inhibition of the latter, or removal of EGF, was paralleled by reductions in phosphorylated ERK1 and 2. Interestingly, suppression of ERK activities with the MEK inhibitor PD184352 mimicked the ability of EGFR inhibitors to enhance the induction of *CYP1A1* by TCDD. Several studies have reported that treatment of cultured cells with U0126 alone (i.e., an MEK inhibitor) is sufficient to induce the accumulation of *CYP1A1* mRNA (Andrieux et al., 2004; Chen et al., 2005; Bachleda and Dvorak, 2008) but also to modestly suppress *CYP1A1* induction when combined with AhR agonists (Andrieux et al., 2004; Chen et al., 2005). In agreement with this latter finding, nonpharmacological modulation of ERK activities in murine Hepa-1 cells indicated that ERKs enhanced TCDD-initiated AhR transcriptional responses (Tan et al., 2004). The current study does not rigorously address if ERKs are the EGFR downstream effectors responsible for dampening AhR function in the MCF10A cell line. However, as demonstrated in Fig. 7, PD184352-mediated suppression of ERK activity had no effect on *CYP1A1* induction in MCF7-TETon-EGFR cultures. Hence, in this latter cell line ERKs are not a regulator of AhR function. Collectively, our data and the existing literature suggest that the relationship between ERK activities and AhR function is most probably cell type- and context-dependent.

The physiologic significance of EGFR-mediated dampening of AhR signaling at this point is speculative but may be relevant to chemotherapy. A variety of therapeutics are metabolized by cytochrome P450s (P450s) (Patterson and Murray, 2002). For example, CYP1A1 and/or 1A2 are involved in the metabolic inactivation of tamoxifen (to a minor extent) and gefitinib (Crewe et al., 2002; Alfieri et al., 2011), and dacarbazine is activated by the same two P450s (Reid et al., 1999). Conditions that would suppress/dampen expression of these P450s appear to be tightly coupled to EGFR activity in MCF10A cells, as pharmacological inhibition of the latter, or removal of EGF, was paralleled by reductions in phosphorylated ERK1 and 2. In contrast, pharmacological inhibition of the Ras/RAF/MEK/ERK pathway was not rigorously addressed if ERKs are the EGFR downstream effectors responsible for dampening AhR function in the MCF10A cell line. However, as demonstrated in Fig. 7, PD184352-mediated suppression of ERK activity had no effect on *CYP1A1* induction in MCF7-TETon-EGFR cultures. Hence, in this latter cell line ERKs are not a regulator of AhR function. Collectively, our data and the existing literature suggest that the relationship between ERK activities and AhR function is most probably cell type- and context-dependent.

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An unexpected outcome of the current study was the absence of a quantitative relationship between CYP1A1 mRNA and protein content in MCF10A cultures. Although TCDD increased CYP1A1 mRNA content more than 50-fold, CYP1A1 protein content increased by less than 3-fold. Furthermore, although cotreatment with EGFR inhibitors and TCDD transiently increased CYP1A1 mRNA content by as much as 3- to 18-fold. Similarly, Alfieri et al. (2011) noted that the addition of EGF to cultured human hepatocytes resulted in a 5- to 10-fold increase in the basal expression of CYP1A1. Similar results were obtained in two additional independent experiments.

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**Fig. 7.** Effects of EGFR expression on *CYP1A1* induction in MCF7-TETon-EGFR cultures. Cultures of MCF7-TETon-EGFR cells were cultured in the presence of 10 nM EGF ± doxycycline. Cultures were left untreated, or exposed to 15 nM TCDD, 250 nM gefitinib, 100 nM Cl-1033, or 250 nM PD184352 ± TCDD. Inhibitors were added 0.5 hours before TCDD and cultures were harvested at the indicated times after TCDD addition for (A) Western blot analyses of EGFR and ERKs, or (B) Northern blot analyses of CYP1A1 mRNA content. Western blot analyses used 25 μg of protein per lane. Results similar to those reported in the figure were obtained in two additional independent experiments.
as ~400-fold, CYP1A1 protein content remained similar to that achieved with TCDD alone. The basis for these findings is not known but is not unique to our study. Two groups have reported markedly discordant relationships between activity levels and CYP1A1 mRNA and protein contents following treatment of MCF10A cultures with TCDD (Chen et al., 2004) or benzo[a]pyrene (Kang et al., 2011). In the case of the mammary carcinoma cell line MCF-7, Divi et al. (2014) reported a 3.79-fold increase in CYP1A1 mRNA content but only an 8.45-fold increase in CYP1A1 protein following exposure to benzo[a]pyrene. Indeed, several studies have reported that mRNA content is not always predictive of protein content for a variety of proteins (Anderson and Seilhamer, 1997; Chen et al., 2002; Lichtinghagen et al., 2002).

**Authorship Contributions**

**Participated in research design:** Joiaikim, Mathieu, Shelp, Boerner, Reiners, Jr.

**Conducted experiments:** Joiaikim, Mathieu, Shelp, and Boerner

**Contributed new reagents or analytic tools:** Boerner

**Performed data analyses:** Shelp, Reiners, Jr.

**Wrote or contributed to the writing of the manuscript:** Mathieu, Reiners, Jr.

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