EXPRESSION PROFILING OF TCDD-TREATED HUMAN EPIDERMAL CELLS IN CULTURE

Miguel A. Rea[1], Jeff P. Gregg[1], and Robert H. Rice2

[1]Department of Medical Pathology and [2]Department of Environmental Toxicology, University of California, Davis, One Shields Avenue, Davis CA, 95616, USA

Introduction

We have previously reported on the modulation of the epidermal growth factor (EGF) effect on telomerase activity by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)[1] and on the modulation of TCDD action on keratinocyte differentiation and morphology by EGF[2]. Here, after microarray expression profiling (~12,000 transcripts) of spontaneously immortalized human keratinocytes (SIK) treated with EGF, TCDD, or both, we report on the effects of TCDD on epidermal cell transcription. Present results provide another example of modulation of TCDD effects. A group of immunity related transcripts belonging to the major histocompatibility complex class II family of genes (MHC-II) depend on the presence of both EGF and TCDD for their expression, whereas treatment with EGF or TCDD alone is ineffective. Properly regulated expression of MHC-II molecules is important for immune function, whereas deficient expression could lead to immunosuppression, and over-expression could lead to an autoimmune state. In addition, proline oxidase, an enzyme required for p53 dependent apoptosis, was identified as a novel TCDD target. Thus, apoptosis suppression though proline oxidase inhibition could contribute to TCDD's tumor promoting effects, and the effect of TCDD on MHC-II could help understand the interaction of TCDD with growth factors such as EGF in perturbing immunity.

Materials & Methods

Cell culture

Cells used were spontaneously immortalized keratinocyes (SIK)[3] passage 40. Cell culture was performed using a feeder layer of lethally irradiated 3T3 cells in a (3:1) mixture of Dulbecco-Vogt Eagle's modified and Ham's F12 media containing 5 % fetal bovine serum, 5 µg/ml transferrin, 5 µg/ul insulin, 0.18 mM adenine, 20 pM triiodothyronine, 0.4 µg/ml hydrocortisone, 10 ng/ml cholera toxin, and antibiotics[4].

TCDD and EGF treatment

When indicated cells were treated with either TCDD (10 nM, sufficient for receptor mediated effects), EGF (10 ng/ml) or both starting at the first medium change, usually three days after inoculation. Treatment continued for 10 days, at which time the cultures were ~80 % confluent, and then total RNA was isolated using phenol/guanidine isothiocyanate/chloroform[5].

Expression profiling

Double stranded cDNA was synthesized starting with 10 mg of total RNA and oligo $(dT)_{24}T7$ primers. Then, biotin-labeled cRNA was synthesized using T7 RNA polymerase. Fragmented cRNA (15 mg) was hybridized overnight (45 °C) to U95Av2 arrays (Affymetrix, Santa Clara, CA). Hybridization was detected with streptavidin-labeled phycoerythrin and confocal laser scanning. The

total intensity of each array was scaled to normalize for inter-array variations. Expression data was generated using Affymetrix MAS 5.0.

Results and Discussion

Long term (10 days) TCDD treatment had a significant effect on epidermal cell transcription, noticeable mostly as gene induction rather than suppression (67 % vs 33 % of the significant changes). Table I lists transcripts whose expression was significantly induced or suppressed by TCDD or EGF/ TCDD treatment. TCDD affected the expression of a variety of gene products including structural proteins, proto-oncogenes, tumor suppressor genes, protease inhibitors, growth factor-related genes, and metabolizing enzymes, among others. Most noticeable among epidermal genes induced by TCDD were CYP1A1 (360 fold), cyclooxygenase-2 (~240 fold), sprIb and II (18 and 8 fold respectively), calcium binding protein A8 (14 fold), and several transcripts related to interleukin 1 (Type II IL-1 receptor, 7 to 26 fold; IL-1 receptor agonist, 4 fold; IL-1b, ~4 fold). Notable among suppressed transcripts were those of proline dehydrogenase/oxidase I (~8 fold) and serine/threonine kinase 19 (~7 fold).

Cyclooxygenase-2 (Cox-2) induction by TCDD agrees with other reports. Indeed, the Cox-2 gene contains a dioxin responsive element (DRE). Interestingly Cox-2 overexpression has been detected in various cancers and a relationship between tumor growth, poor prognosis, and Cox-2 overexpression has been noted. Cox-2 directly affects production of prostaglandins and other mediators of inflammation, promotes angiogenesis, and inhibits apoptosis[6]. Thus, continued Cox-2 induction by TCDD could be instrumental in TCDD's tumor promotion effects.

TCDD induction of IL-1 related genes could be important in orchestrating TCDD effects. It is known, for instance, that IL-1b is the major Cox-2 inducer in the CNS[7] and that the IL-1 receptor antagonist IL-1RA competitively binds the IL-1 receptor and neutralizes the effects of IL-1 a and b, thus down regulating immune response[8]. Although physiological IL-1 receptor inhibition by IL-1RA is beneficial in controlling exaggerated immune responses, in non-physiological conditions it could lead to immunosuppression, a well known effect of TCDD.

Proline oxidase is a mitochondrial enzyme central in the proline/pyrroline-5-carboxylate redox cycle. It catalyzes the conversion of proline to pyrroline-5-carboxylate (P5C), and is ultimately involved in the transfer of redox potential across the mitochondrial membrane. Developmental functions of this enzyme can be inferred in cases of this enzyme deficiency[9]. Furthermore, proline oxidase has been shown to increase in cells undergoing p53 dependent apoptosis, and P5C, its end metabolite, has been shown to suppresses cell growth and induce apoptosis[10]. Suppression of proline oxidase by TCDD could explain its observed ability to inhibit programmed cell death.

CYP1A1 and CYP1B1 are normally absent in cultured keratinocytes, but their expression is activated by certain aromatic hydrocarbons. Indeed, TCDD treatment of epidermal cells highly stimulates CYP1A1 transcription by 362 fold (Table II). It stimulates CYP1B1 transcription as well, but to a lesser extent (~5.3 fold). The Presence of EGF does not alter TCDD induced P450 expression. Although not stimulated by TCDD treatment, but equally toxicologically relevant, cultured keratinocytes express CYP4B1, CYP2D6, CYP11A1, CYP21A1P, and CYP4F3. This information is consistent with the low level expression of a number of cytochromes P450 in epidermis that have been difficult to characterize, although detection of some of these forms by PCR has been reported[11]. Present results are of interest for understanding the response to pharmaceuticals applied to skin for local or systemic therapy.

Table 1. Differentially expressed genes in human epidermal cells (SIK) by TCDD and EGF/TCDD treatment

	DMSO	TCDD vs. DMSO			EGF/TCDD vs. DMSO		
Description	*Signal*	*Signal*	*Fold change (95% Conf. Int.)*	*Change P value*	*Signal*	*Fold change (95% Conf. Int.)*	*Change P value*
Induction	†						
CYP1A1	A	4234	362 (128 , 1098)	0	5491	446 (158 , 1352)	0
Cyclooxygenase-2 (hCox-2)	A	425	239 (91 , 630)	0	409	208 (74 , 588)	1E-06
Small proline rich protein IB (sprIb)	A	576	18.4 (9.2 , 36.8)	0	306	9.2 (6.5 , 13.9)	0
S100 calcium binding prot A8 (S100A8)	A	572	13.9 (6.1 , 34.3)	1E-06	655	13.9 (5.3 , 36.8)	1E-06
Aldehyde dehydrogenase (ALDH1A3)	A	1282	13 (9.2 , 17.2)	0	1320	12.1 (9.2 , 16)	0
Cold shock domain protein A (CSDA)	A	567	10.6 (6.1 , 17.2)	0	744	12.1 (6.5 , 22.6)	0
Serine proteinase inhibitor (SERPINB2)	117	913	8.6 (6.5 , 10.6)	1E-06	801	7.5 (6.1 , 9.9)	0
Small proline rich protein (sprII)	A	527	8 (2.8 , 22.7)	1E-06	597	8.6 (2.8 , 26)	4E-06
SERPINE1 serine proteinase inhibitor	A	290	8 (5.7 , 12.1)	0	411	10.6 (7.5 , 16)	0
Type II interleukin-1 receptor	A	228	7.5 (4 , 13.9)	7,9E-05	493	26 (8 , 79)	1E-06
MOT8 Hypothetical protein	A	132	7.5 (3.7 , 14.9)	0	A	0	0
Thrombospondin 2	A	183	7 (3.7 , 13)	1,1E-05	150	6.5 (3.7 , 10.6)	1E-06
Amphiregulin (AR)	157	839	6.5 (5.3 , 8)	1E-06	728	5.7 (4.6 , 6.5)	0
beta-galactoside-binding lectin	171	1466	8.6 (7 , 9.9)	4E-06	1146	6.1 (4.3 , 8.6)	4E-06
S100 calcium binding protein A9 (S100A9)	146	709	6.1 (4.9 , 7.5)	1E-06	963	7.0 (5.3 , 9.2)	1E-06
Fucosidase, alpha-L- 1 (FUCA1)	229	2154	5.3 (4.3 , 6.1)	0	3622	8.0 (6.1 , 9.9)	0
IMAGE clone 1420488	A	457	5.3 (3.5 , 8)	0	1257	17.2 (12.1 , 22.6)	0
Leukemia virus receptor 1 (GLVR1)	240	1115	4.6 (3 , 7.5)	0	1095	4.3 (3.1 , 6.1)	1E-06
Vascular endothelial growth factor (VEGF)	A	368	4.6 (4 , 5.3)	0	411	4.9 (4 , 5.7)	0
V-maf oncogene (MAFF)	A	286	4.6 (4 , 5.7)	0	395	7 (6.1 , 8)	0
DKFZP434J214 DKFZP434J214 protein	A	177	4.6 (3.5 , 6.5)	0	173	3.7 (2.8 , 4.9)	0
Pyrimidinergic receptor P2Y (P2RY6)	A	251	4.3 (3 , 5.7)	2E-06	372	6.5 (4.6 , 8.6)	2E-06
Interleukin 1 receptor antagonist (IL1RN)	224	792	4.0 (3 , 4.9)	0	800	4 (3.3 , 4.9)	0
Laminin, gamma 2 (LAMC2)	A	266	4.0 (3.3 , 4.9)	0	275	3.7 (3 , 4.6)	1E-06
Thyroid hormone binding protein (p55)	A	294	3.7 (2.3 , 5.7)	2E-06	219	3.0 (2.3 , 4)	1E-06
Interleukin 1, beta (IL1B)	A	278	3.7 (2.1 , 7)	0	406	5.7 (2.8 , 11.3)	2E-06
F3 Coagulation factor III	A	201	3.7 (3 , 4.6)	0	128	0	2E-06
Lipocalin 2 (LCN2, oncogene 24p3)	620	2093	3.5 (2.8 , 4)	1E-06	3018	5.3 (4.3 , 6.5)	0
Transforming growth factor-beta	391	1351	3.5 (3 , 3.7)	0	1556	4 (3.5 , 4.3)	0
Laminin, alpha 3 (LAMA3)	218	718	3.5 (2.8 , 4)	0	595	3.0 (2.5 , 3.7)	0
cDNA DKFZp586C1619	A	226	3.5 (2.1 , 5.7)	2E-06	250	3.5 (2.1 , 5.3)	0
UDP-glucose ceramide glucosyltransferase	A	143	3.5 (2.8 , 4.3)	3E-06	128	0	0
Keratin 16 (KRT16)	570	1238	3.3 (2.5 , 4)	1E-06	793	2.3 (2 , 2.6)	0,00047
Angiopoietin-like 2 (ANGPTL2)	A	223	3.3 (2 , 5.3)	1,2E-05	250	4 (2.6 , 5.7)	9E-06
Oncostatin-M	A	180	3.3 (3 , 3.7)	0	255	3.7 (3.3 , 4.6)	0
Supression							
Proline dehydrogenase/oxidase 1 (PRODH)	242	A	-7.5 (-18.4 , -3)	4E-05	A	-3.3 (-5.3 , -2.1)	5,4E-05
Serine/threonine kinase 19 (STK19)	181	A	-7.0 (-18.4 , -2.6)	1,4E-05	A	-8 (-19.7 , -3.5)	7E-06
Anterior gradient 2 homolog (AGR2)	326	A	-7 (-9.2 , -5.7)	1E-06	A	-8 (-10.6 , -6.1)	1E-06
DKFZp586A0522	349	A	-5.3 (-6.1 , -4.3)	0	A	-4.3 (-5.3 , -3.5)	0
Keratin 4 (KRT4)	1668	294	-4.9 (-9.9 , -2.5)	1E-06	A	-5.7 (-12.1 , -2.6)	3E-06
Collagen, type IX, alpha 3 (COL9A3)	325	A	-3.7 (-4.3 , -3.3)	1E-06	A	-7.0 (-10.6 , -4.9)	1E-06
Keratin 15 (KRT15)	745	171	-3.7 (-5.7 , -2.7)	0	135	-3.5 (-4.3 , -2.6)	0
Selenium-binding protein (hSBP)	844	146	-3.5 (-5.3 , -2.3)	3E-06	247	-2.5 (-3.5 , -1.6)	5E-06
E48 Lymphocyte antigen 6 complex, locus D	850	355	-3.3 (-4.9 , -2.3)	0	452	-2.5 (-3.5 , -1.9)	0
Phosphoenolpyruvate carboxykinase	219	A	-3.3 (-4.6 , -2.1)	4E-06	213	0	0,74359
Inhibitor of DNA binding 3	2418	650	-3.3 (-4 , -2.6)	0	451	-3.7 (-5.7 , -2.5)	0
Actin binding LIM protein (ABLIM)	222	A	-3.0 (-4.3 , -2.3)	9,2E-05	A	-4.9 (-11.3 , -2.1)	2,5E-05
Milk fat globule-EGF factor 8 protein	146	A	-3.0 (-4.3 , -2.1)	1,2E-05	A	-3.7 (-6.5 , -2.14)	5,9E-05
Biglycan (BGN)	896	252	-2.8 (-3.7 , -2.3)	0	278	-2.6 (-3.3 , -2.1)	0
Clusterin SP-40 (CLU)	1709	672	-2.6 (-2.8 , -2.5)	0	450	-3.48 (-4 , -3.03)	0
Ataxia-telangiectasia group D-associated protein	1294	475	-2.5 (-2.8 , -2.3)	1E-06	460	-2.64 (-2.83 , -2.3)	0
Biliverdin reductase B (BLVRB)	1118	478	-2.3 (-2.5 , -2.3)	1E-06	625	-1.23 (-1.3 , -1.15)	1E-06
Argininosuccinate synthetase (ASS)	3250	1211	-2.3 (-2.6 , -2)	0	2447	-1.23 (-1.3 , -1.15)	1E-06

Induced transcripts had a lower limit 95% confidence interval ≥ 2. Suppressed transcripts had an upper limit 95 % confidence interval ≤ -2. †A, absent (signal ≤ 100 or detection P value ≥ 0.06)

Table 2. Cytochrome P450 genes expressed in human epidermal cells and their induction by TCDD

	DMSO		TCDD vs. DMSO			
Isozyme	*Signal*	*Detection P value*	*Signal*	*Detection P value*	*Fold change (95% Conf. Int.)*	*Change P value*
CYP1A1	8,6	0,34	4234,2	0,0002	362 (128 - 1098)	0
CYP4B1	226,7	0,02	205,0	0,06	0.93 (0.81 - 1)	0,5
CYP2D6	151,3	0,05	189,1	0,04	1.15 (1 - 1.32)	0,5
CYP11A1	107,8	0,02	162,4	0,02	1.15 (1 - 1.32)	0,5
CYP21A1P	119,0	0,08	143,7	0,07	1.07 (0.93 - 1.15)	0,5
CYP1B1	17,5	0,10	106,6	0,0008	5.28 (3.03 - 9.19)	1E-06
CYP4F3	108,4	0,01	91,1	0,04	1 (0.87 - 1.07)	0,5
CYP2A7	64,0	0,10	62,4	0,05	1.07 (0.93 - 1.23)	0,5
CYP2E1	21,8	0,11	38,6	0,03	1.62 (1.32 - 2)	0,02
CYP2C9	18,2	0,11	31,7	0,02	1.07 (0.87 - 1.41)	0,5
CYP4F2	10,5	0,22	22,4	0,04	1.41 (1 - 2.14)	0,5
CYP51	15,9	0,01	17,1	0,003	0.81 (0.47 - 1.52)	0,3

Although similar transcriptional effects were observed in epidermal cells treated with TCDD alone and in those co-treated with EGF and TCDD (see Table I), a group of transcripts was observed to be significantly induced only when epidermal cells were concurrently treated with EGF and TCDD (Table III). Nearly 60 % of those transcripts (labeled with *) belong to the MHC-II family of genes. Antigen presenting cells are normally the only cells that express MHC-II proteins on their surfaces. MHC-II molecules are heterodimeric (α/ß) glycoproteins belonging to one of three isotypes (HLA-DR, HLR-DQ, and HLR-DP). Different isotypes of both a and b polypeptides are induced by EGF/TCDD treatment (Table III). MHC-II molecules affected by EGF/TCDD are involved in a/b complex stabilization, MHC-II trafficking, antigen loading, and peptide digestion. The orchestrated induction by TCDD/EGF of MHC-II molecules suggests that a common repressor or inducer of MHC-II expression has been affected by the treatment.

Table 3. Transcripts found differentially expressed only with concurrent EGF and TCDD treatment

			DMSO	EGF/TCDD vs DMSO		
Description	*Genbank*	*Unigene*	*Signal*	*Signal*	*Fold change (95% Conf. Int.)*	*Change P value*
* HLA-DR MHC class II, beta-1	M32578	Hs.181366	†A	179	194 (56 - 676)	0
HSD17B2 Hydroxysteroid (17-beta) dehydrogenase 2	L40802	Hs.155109	A	174	52 (16 - 169)	1E-06
* HLA-DQB1 class II, DQ beta 1	M60028	Hs.73931	A	121	13.9 (4.9 - 42.2)	5,4E-05
* HLA-DRA class II, DR alpha	J00194	Hs.76807	A	668	8.6 (6.5 - 12.1)	0
* CD74 CD74 antigen (invariant polypeptide of MHC)	M13560	Hs.84298	A	491	8 (4.9 - 12.1)	0
* HLA-DPA1 MHC, class II, DP alpha 1	X00457	Hs.914	A	403	5.7 (2.1 - 16)	8E-06
* C1S Complement component 1, s subcomponent	J04080	Hs.169756	A	140	5.3 (2.8 - 9.9)	9E-06
Fgr proto-oncogene	M19722	Hs,1422	A	539	4.9 (3.0 - 8.6)	1E-06
* HLA-DMA MHC, class II, DM alpha	X62744	Hs.77522	A	376	4 (3.0 - 5.3)	0
* PSMB9 Proteasome (prosome, macropain)	AA808961	Hs.9280	182	661	3.5 (2.6 - 4.6)	0
Folate receptor alpha	U78793	Not Aval.	A	232	3.5 (2.5 - 4.9)	2E-06
Ras-related associated with diabetes (RRAD)	L24564	Hs.1027	A	222	3.5 (2.3 - 4.9)	0
Non-functional folate binding protein	AF000381	Hs.239816	A	187	3.0 (2.3 - 4)	8E-06
* Chymotrypsin-like protease MECL-1	X71874	Hs.9661	328	692	2.8 (2.3 - 3.5)	0
Transglutaminase 2 (TGM2)	M55153	Hs.8265	274	938	2.8 (2.6 - 3.3)	0
Retinol binding protein 1 (RBP1)	M11433	Hs.101850	A	146	2.8 (2.1 - 4)	2E-06
* HLA-DR7-associated glycoprotein MHC class II	M16941	Hs.180255	206	556	2.63 (2 - 3.48)	0
* BF B-factor, properdin	L15702	Hs.69771	A	238	2.46 (2 - 3.25)	0
DNA-binding protein (AP-2)	M36711	Hs.18387	437	868	2.46 (2.14 - 2.83)	0
Apolipoprotein L, 1 (APOL1)	AF019225	Hs.114309	A	227	2.29 (2 - 2.46)	0

* Related to MHC-II proteins

†A, absent (signal ≤ 100 or detection P value ≥ 0.06)

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