AhR/IL-24 signaling is associated with susceptibility to dioxins

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Background:

Dioxins are a class of highly toxic and persistent environmental pollutants that cause multiple adverse health effects in humans, mainly through binding to the ligand-activated transcription factor, aryl hydrocarbon receptor (AhR). Genetic variation in AhR may modulate the susceptibility to dioxins, and little is known about the downstream signaling pathways that lead to multiple adverse health effects.

Objectives:

In this study, we aimed to evaluate the effects of the single nucleotide polymorphism (SNP) –130C/T in the AhR promoter on dioxin-inducible gene transcription, and to investigate downstream signaling pathway associated with susceptibility to dioxins.

Methods:

Cells were isolated from normal human chorionic villi and genotyped by PCR-RFLP. The gene expression profiles were assessed using cDNA microarray after exposure of cells with 10nM for 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) for 24h. Differentially expressed genes were further validated by real-time RT-PCR and Western blotting. Blood samples from 64 Yusho patients who were accidentally exposed to high concentrations of dioxins were analyzed for the genotype. Serum dioxins concentrations and cytokine concentrations were detected by using high-resolution gas chromatography/high-resolution mas spectrometry and enzyme-linked immunosorbent assay, respectively. Multiple liner regression models were performed to examine the association between serum cytokine levels and dioxins levels in Yusho patients' blood.

AhR SNP -130 C/T regulates the expression of AhR in normal human chorionic stromal cells

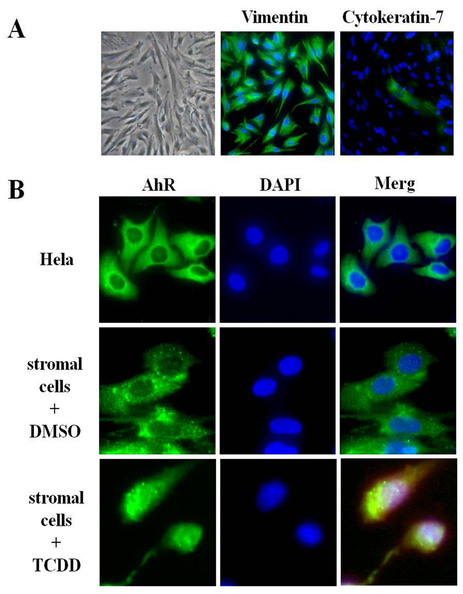


Figure 1. Identification of normal human chorionic stromal cells and distribution of endogenous AhR in cells. (A) Most cells were vimentin-positive (middle), but only a few cells were cytokeratin 7-positive (right). (B) AhR was localized prominently in the cytoplasm of both HeLa cells and normal human villous stromal cells. After treatment of stromal cells with TCDD, AhR was translocated into nucleus.

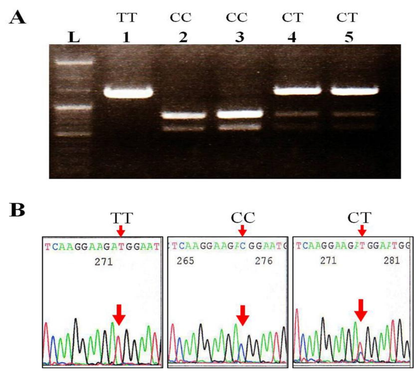


Figure 2. Investigation of AhR genotype using PCR-PRLP electrophoresis (A) and PCR-sequencing (B).

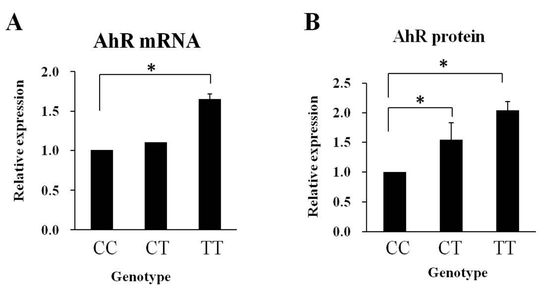


Figure 3. Expression levels of AhR mRNA (A) and protein (B) in normal human chorionic stromal cells with AhR –130 CC, CT, or TT genotype (CC: n = 4; CT: n = 4; TT: n = 3). The data are representative of three independent experiments (*p < 0.05).

Gene expression profiles of human chorionic stromal cells exposed to TCDD

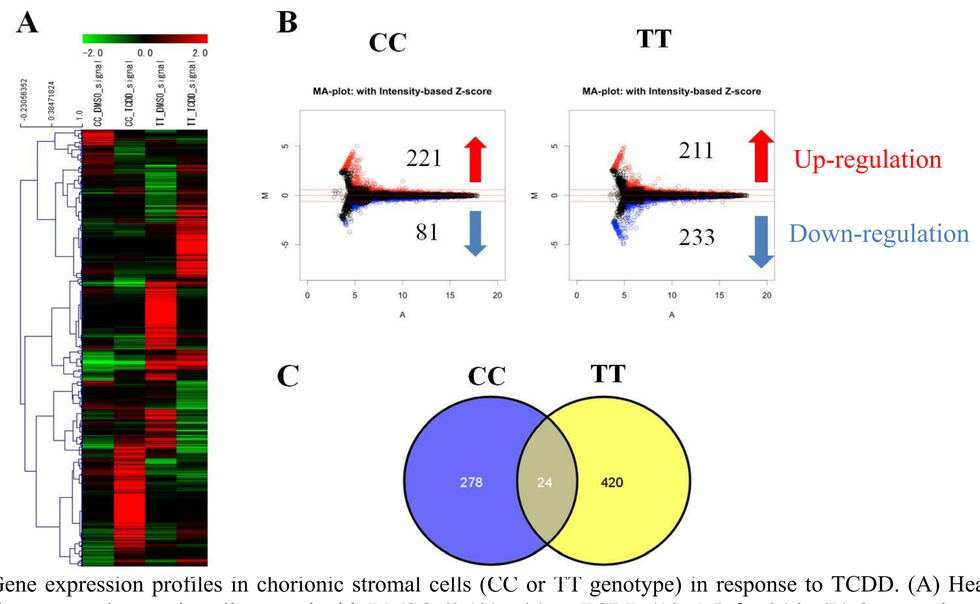


Figure 4. Gene expression profiles in chorionic stromal cells (CC or TT genotype) in response to TCDD. (A) Heat map of differentially expressed genes in cells treated with DMSO (0.1%, v/v) or TCDD (10 nM) for 24 h. (B) Scatter plot presenting the up-regulated and down-regulated genes in response to TCDD for the CC (left) and TT (right) genotype. (C) Venn diagrams presenting 24 genes for which the expression pattern in response to TCDD was similar between the CC and TT genotypes.

Validation of genes differentially expressed in response to TCDD

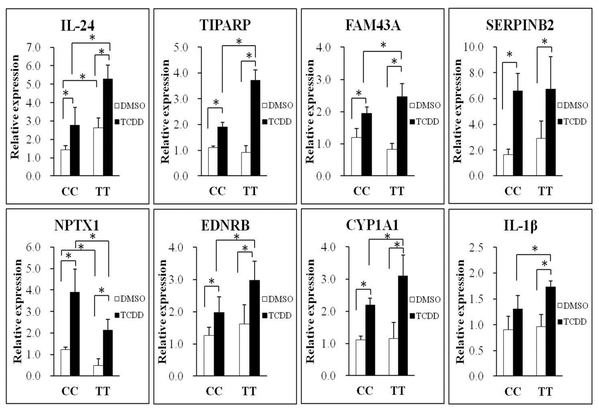


Figure 5. Validation of gene expression by using real-time RT-PCR. Chorionic stromal cells (CC: n = 6; TT: n = 3) were treated with DMSO (0.1%, v/v) or 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) (10 nM) for 24 h. The data show mRNA levels (normalized to *GAPDH*) relative to DMSO-treated controls with the CC genotype. The data are representative of five independent experiments (*p < 0.05).

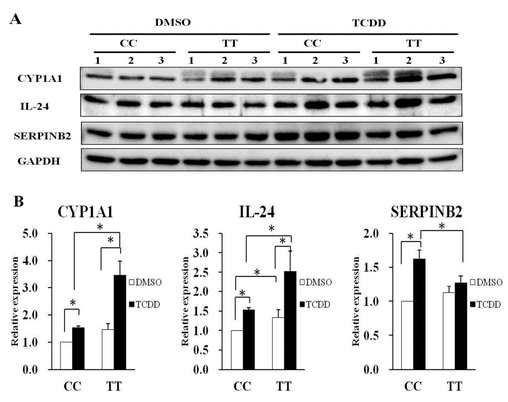


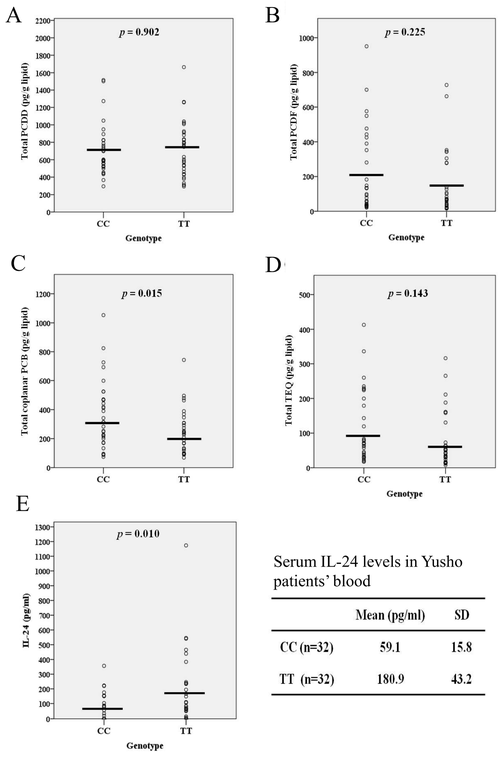
Figure 6. Validation of gene expression by using western blotting. Chorionic stromal cells (CC: n = 3; TT: n = 3) were treated with DMSO (0.1%, v/v) or TCDD (10 nM) for 48 h. (A) Western blotting showing protein expression. (B) Protein expression level of CYP1A1, IL-24, and SERPINB2 (normalized to GAPDH) in TCDD-treated cells. The data are representative of three independent experiments (*p < 0.05).

Dioxins concentrations and serum IL-24 levels in Yusho patients' blood

Table 1 Characteristics of study subjects

Characteristics	Number (%)		p value [d]
	CC genotype	TT genotye	
Patients	32	32	
Gender			0.217
Male	14 (43.8%)	19 (59.4%)	
Female	18 (56.4%)	13 (40.6%)	
Age at blood sampling (years)			0.672
40-50	3 (9.4%)	1 (3.1%)	
50-60	2 (6.3%)	9 (28.1%)	
60-70	9 (28.1%)	4 (12.5%)	
70-80	9 (28.1%)	8 (25%)	
80-90	9 (28.1%)	10 (31.3%)	
Age at exposure (years)			0.656
0-10	5 (15.6%)	8 (25%)	
10-20	5 (15.6%)	3 (9.4%)	
20-30	10 (31.3%)	9 (28.1%)	
30-45	12 (37.5%)	12 (37.5%)	
Interval between exposure and blood sampling (years)	42.3 ± 0.9 [a]	42.2 ±1.3 [a]	0.826
Interval between cytokine detection and dioxin detection (years)	1.0 ± 0.7 [a]	1.3 ±1.0 [a]	0.087
Smoking status			0.172
Smoking	2 (6.3%)	6 (18.8%)	
Non-smoking	23 (71.9%)	21 (65.6%)	
Others [b]	7 (21.9%)	5 (15.6%)	
Frequency of seafood consumption			0.596
Every day	2 (6.3%)	8 (25%)	
3-4 times/week	15 (46.9%)	9 (28.1%)	
1-2 times/week	11 (34.4%)	10 (31.3%)	
Others [c]	4 (12.5%)	5 (15.6%)	

[a] Mean ± standard deviation
[b] Patients who quit smoking
[c] Patients who consume seafood a maximum of once or twice a month
[d] Student's t-test



Serum IL-24 levels in Yusho patients' blood

	Mean (pg/ml)	SD
CC (n=32)	59.1	15.8
TT (n=32)	180.9	43.2

Figure 7. Dioxins concentration and serum IL-24 levels in Yusho patients' blood. Serum total coplanar PCB concentrations were significantly higher for the CC genotype than for the TT genotype. The serum IL-24 level for the TT genotype was significantly higher (p = 0.010) than that for the CC genotype.

Serum dioxins concentrations are not correlated with serum IL-24 levels in Yusho patients

Table 2. Association of serum IL-24 with blood levels of total PCDD, total PCDF, total coplanar PCB, and total TEQ in Yusho patients.

	Total (n=64)				C/C (n=32)				T/T (n=32)			
	Beta	95%CI		p	Beta	95%CI		p	Beta	95%CI		p
Total PCDDs	118.7	-252.9	490.3	0.524	-14.1	-233.8	205.6	0.895	331.8	-420.4	1084.1	0.369
Total PCDFs	-55.3	-764.1	653.6	0.876	-110.6	-563.6	342.4	0.617	16.3	-1515.7	1548.4	0.983
Total coplanar PCBs	189.7	-224.4	603.8	0.362	334.7	84.2	585.2	0.011	-81.4	-1022.8	860.1	0.859
Total TEQ	-0.3	-1027.4	1026.8	1.000	-105.8	-788.4	576.9	0.750	231.2	-1913.9	2376.3	0.825

Results were calculated as multiple linear regression models adjusted for gender, exposure years, interval between exposure and blood sampling, interval between cytokine detection and dioxin detection, smocking status and seafood consumption.

Conclusion

In the present study, we demonstrated that AhR SNP –130C/T modulates AhR mRNA and protein expression in normal human chorionic stromal cells. We provide a list of potential AhR target genes that affect outcome after TCDD exposure. In particular, we found that IL-24, which is associated with the inflammatory response, acts as an AhR downstream effector. AhR SNP –130C/T affects serum IL-24 levels independently of serum dioxins concentrations in Yusho patients. Our preliminary results suggest a possible association of AhR genotype with inflammatory disease including pollinosis, asthma and so on, of which incidences are higher in Yusho patients with the TT genotype than those with the CC genotype (unpublished data). These results indicates that AhR/IL-24 signaling is associated with susceptibility to dioxins.

Our investigation provides new insights into the understanding of the mechanisms of health impairments in Yusho patients and genetic susceptibility to dioxins. Investigation of the mechanism of AhR/IL-24 signaling in pathogenesis of dioxin-induced health damage is required. Further longitudinal cohort studies should be carried out to confirm our findings and to understand the adverse health effects in response to dioxins exposure for current and subsequent generations.