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

Ge Liu,1 Kazuo Asanoma,2 Tomoka Takao,1 Kiyomi Tsukimori,1 Hiroshi Uchi,4,5 Masutaka Furue,4,5 Kiyoko Kato,2 Norio Wako†

1Department of Genomic Epidemiology, Research Center for Environment and Developmental Medical Sciences, Kyushu University, Fukuoka, Japan; 2Department of Obstetrics and Gynecology, Graduate School of Medical Science, Kyushu University, Fukuoka, Japan; 3Department of Obstetrics, Fukusuka Children's Hospital, Fukusuka, Japan; 4Research and Clinical Center for Yusho and Dioxins, Kyushu University Hospital, Fukuoka, Japan; 5Department of Dermatology, Graduate School of Medical Science, Kyushu University, Fukuoka, Japan.

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 promote 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 gene. Serum dioxins concentrations and cytokine concentrations were detected by using high-resolution gas chromatography/high-resolution mass 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 stalk cells

AhR mRNAs and AhR proteins were observed in chorionic stalk cells, while AhR proteins were not in chorionic villus cells.

Validation of genes differentially expressed in response to TCDD

Figure 5. Validation of gene expression by using real-time RT-PCR. Chorionic stalk cells (CC: n = 6; TT: n = 3) were treated with DMSO (0 %, v/v) or TCDD (10 nM) for 24h. 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).

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

Gene expression profiles of human chorionic stalk cells exposed to TCDD

Figure 4. Gene expression proteins in chorionic stalk cells (CC or TT genotype) in response to TCDD. (A) Heat map of differentially expressed genes in cells treated with DMSO (0 %, v/v) or TCDD (10 nM) for 24h. (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.

Conclusion
In the present study, we demonstrated that AhR SNP –130C/T modulates AhR mRNA and protein expression in normal human chorionic stalk 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 poliomyelitis, asthma and so on, of which incidences are higher in Yusho patients with the TT genotype than with those with the CC genotype (unpublished data). These results indicate 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.