(第3号様式) (Form No. 3)

学 位 論 文 要 旨
Dissertation Summary

氏　　名 (Name)　Hoa Thanh Nguyen

論　文　名 (Dissertation Title)：Effects of exposure to environmental chemicals on the liver transcriptome and proteome of rodents
環境汚染物質の暴露がげっ歯類の肝トランスクリプトーム・プロテオームに与える影響

Toxicology is a field of science that helps us understand the harmful effects that chemicals, substances, or situations, can have on people, animals, and the environment. With the development in biotechnology, toxicologists have been now trying to uncover the molecular mechanisms of the toxicity of environmentally persistent compounds. "Omics" approaches such as transcriptomics and proteomics are being used more frequently within the field of system toxicology to draw adverse outcome pathways of emerging environmental contaminants. The aim of my studies is to investigate the effects of environment chemicals at the transcriptome and proteome levels in laboratory rodents. The results from these studies may help us to understand the mode of actions of these chemicals with specificity in strains, genders, growth stages, and doses of exposure.

The first study focused on the strain differences in the effects of 2,3,7,8-tetrabromo-dibenzo-p-dioxin (TBDD). TBDD is an analogue of 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD), which is one of the most potent dioxins and dioxin-like-compounds. Studies on the toxicology of TBDD indicated that this compound influences a spectrum of effects likely to that of TCDD in laboratory animals. It has been demonstrated that there are strain- and species-specific differences in the aryl hydrocarbon receptor (AHR)-mediated responses to dioxin exposure. Here, we used the two inbred mouse strains C3H/HeJ-*lpr*/*lpr* (C3H/*lpr*) and MRL/MpJ-*lpr*/*lpr* (MRL/*lpr*) which have different AHR genotypes at the 375th position. C3H/*lpr* AHR presents an Ala residue at the 375th position, which has a high affinity to TCDD, while MRL/*lpr* AHR encodes a Val which has lower affinity to TCDD. Thus, C3H/*lpr* and MRL/*lpr* are considered as sensitive- and resistant-strain, respectively. Here, we adopted a proteomic approach using two-dimensional electrophoresis (2-DE) and matrix-assisted laser desorption/ionization time of flight/time of flight

(MALDI-TOF/TOF) mass spectrometry (MS) to identify the differences in the effects of TBDD exposure on the hepatic proteome between the two mouse strains. To confirm the strain-difference in response to TBDD treatment, cytochrome P450 (CYP) 1A1 and 1A2 protein levels were measured in both strains. A dose of 10 μg/kg body weight of TBDD induced hepatic CYP1A1 and CYP1A2 expression in both strains, but the expression levels of both CYP1A proteins were higher in C3H/*lpr* mice than in MRL/*lpr* mice. The result supports that C3H/*lpr* mice are more sensitive to dioxins than MRL/*lpr* mice. We have successfully identified proteins that were more induced or suppressed by TBDD treatment in C3H/*lpr* mice. These include proteins responsible for AHR activation through production of endogenous ligands such as aspartate aminotransferase, indolethylamine N-methyltransferase, and aldehyde dehydrogenases, as well as reducing oxidative stress, such as superoxide dismutase and peroxiredoxins. Taken together, our results provide insights into the molecular mechanism underlying the high dioxin-susceptibility of the C3H/*lpr* strain, in which AHR activation by TBDD is more prompted by the production of endogenous ligands, but the adaptation to oxidative stress is also acquired.

The second study focused on an emerging environmental contaminant, bisphenol A (BPA). BPA has been known as a major endocrine disrupting chemical which is widely detected in the environment and human. BPA has been detected in body fluids and tissues including umbilical cord blood, human breast milk, amniotic fluid, fetal serum, and placental tissue, suggesting that BPA can be transferred from mother to fetus during pregnancy and lactation. Therefore, there is a growing concern about the potential developmental toxicity of BPA. Studies in developmental exposure demonstrated that BPA has adverse effects on male and female reproduce systems, metabolic homeostasis, and may associate with liver dysfunction, type 2 diabetes, and cardiovascular disorders. These effects may depend on sexes, ages, and dose exposures. The aim of this study is to unveil the mechanism of the transgenerational actions of BPA based on the changes in hepatic transcriptome and proteome in rat offspring. We exposed pregnant Wistar rats to low (50 μg/kg bw/day) and high (5000 μg/kg bw/day) doses of BPA or 17β-estradiol (E2, 50 μg/kg bw/day) from embryonic day 3 to 18. Firstly, the offspring were observed for any changes in their body and organ weights and anogenital distance (AGD) at PND1-2 (newborn), PND21-22 (weaning), and PND59-60 (young). We observed significantly increased body weight and longer AGD in both females and males at some periods. There were also several changes in organ weights. These changes were found differently between sexes, ages, and doses of exposure.

Secondly, we analyzed the hepatic transcriptome profiled of the offspring at newborn and weaning by a paired-end sequencing with Illumina HiSeq2500. The genes which had significantly different FPKM (fragments per kilobase of transcripts per million mapped reads) in BPA- or E2-treated groups from those in the control (vehicle-treated) group (FDR < 0.05) were selected as differentially expressed genes (DEGs) and used for further analyses including biological function and transcription factor analyses. Transcriptome data revealed that there were more changes in newborn rats than in weaning rats. A principal component analysis using FPKMs of all DEGs demonstrated that the prenatal BPA exposure caused the masculinization of the hepatic transcriptome in females at newborn and weaning stages. It suggests antagonistic effects of BPA to endogenous estrogens on female offspring. Transcriptome profile of newborn offspring showed that BPA exposure up-regulated genes related to the cell cycle, but down-regulated genes related to the chemical carcinogenesis in both females and males. In newborn female pups, even the low dose of BPA induced the DNA replication and insulin resistance, and depressed the fatty acid degradation, steroid hormone synthesis, and PPAR signaling

pathways. In newborn male pups, only the high dose of BPA altered gene expressions in the peroxisome, PPAR signaling, fatty acid degradation, and other metabolic pathways. Transcriptome profile of weaning offspring showed that even low dose of BPA changed the expression levels of genes involved in several infectious and metabolic diseases in females, and in metabolic pathways in males. Interestingly, most of the effects found in newborn females were reduced or disappeared at weaning, whereas the effects in newborn males were increased or continued at weaning. These results indicate different modes of action of prenatal BPA exposure between male and female offspring as well as between early and later life stages. We also detected altered expression of epigenetics-related genes including DNA methyl- transferases (DNMTs), histone acetyltransferases (HATs), methyltransferases (HMTs), deacetylases (HDACs), and demethylases (HDMs). These changes were mostly observed in newborn females, suggesting the role of epigenetics in susceptibility to BPA exposure. In addition, BPA was found to induce or inhibit numerous xenobiotic metabolizing enzymes including phase I, II, and III enzymes in postnatal animals, suggesting BPA may have crosstalk with effects of other contaminants and drugs.

Thirdly, we examined the expression levels of liver proteins in newborn pups using a high-throughput approach, isobaric tags for relative and absolute quantitation (iTRAQ) - labelling followed by nano-liquid chromatography (LC) and MALDI MS/MS. Based on the ratios between iTRAQ-tags of treated group and control group (≥ 1.5 or ≤ 0.7), we decided the differentially expressed proteins (DEPs) by each treatment and used them for biological function and network analyses. Proteomic data also showed differences between female and male pups. We also identified DEPs involved in lipid and steroid hormone metabolism. Together with transcriptomic data, these data suggest that prenatal exposure to BPA induces lipid accumulation and imbalance of sex hormones, consequently lead to an increase of body weight and AGD length in offspring. Network analysis of the DEPs indicated several hubs including CPS1, GADPH, TXN1, ALB, and ACTB, which may be considerable as genetic makers for the developmental effects of BPA.

Overall, the studies in this dissertation demonstrated a valuable strategy to clarify the molecular mechanisms of genetic (strain)-, sex-, age-, and dose-dependent effects of environmental contaminants.