Toxicogenomic Study for Mechanism of Action of Methylmercury in Human Neuronal Cells

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Methylmercury (MeHg) is a significant environmental contaminant that will continue to pose great risk to human health. Increasing evidences indicate that MeHg is a significant threat to a variety of organ systems including the brain and central nervous system. However, little is known about the neurotoxic mechanism or molecular target of MeHg in human neuronal cells. In this respect, a strategy which aims at identifying sensitive molecular targets of MeHg at environmentally relevant levels may prove particularly useful to understanding of mechanisms and risk assessment. In this thesis, toxicogenomic study was attempted to provide information regarding the mechanisms and the possible marker genes on its complicated cellular response to neurotoxicity of MeHg.

First, we investigated the analysis of transcriptional profiles by treatment of 1.4μM MeHg for 3, 12, 24 and 48 h in human neuroblastoma SH-SY5Y cell line. Some of the identified genes by MeHg treatment were significant at early time points (3 h), while that of others was at late time points (48 h). This result suggests that a neurotoxic effect of the MeHg might be ascribed that MeHg alters NMDA receptor regulation or homeostasis of neuronal cells in the early phase. However, in the late phase, it protects cells from neurotoxic effects of MeHg.

Secondly, we shown that exposure to MeHg significantly impairs viability and mRNA expression of selenoprotein W (SeW) with dose- and time- dependent manner, unlike other selenoenzymes, such as selenoprotein P, glutathione peroxidase, iodothyronine 5-deiodinase, iodothyronine 5'-deiodinase. Furthermore, a positive correlation was found between SeW mRNA level and intracellular glutathione (GSH) but any significant correlation was not observed between intracellular reactive oxygen species (ROS) and SeW mRNA level or intracellular GSH contents. Therefore, we suggest that SeW is the novel molecular target of MeHg in human neuronal cells, and down-regulation of this selenoenzyme by MeHg is dependent on but depletion of GSH not generation of ROS.

Thirdly, observations of greater neurotoxicity with fetal compared with adult exposure suggest a unique susceptibility of the developing nervous system to MeHg. To determine definitive molecular mechanisms underlying the neurotoxic effects of MeHg in developing nervous system, differentiating and differentiated SH-SY5Y neuronal cell models were employed in this study. In differentiating and differentiated cells, following 48 h exposure, 1.8μM MeHg significantly decreased retinoic acid (RA)-stimulated neurite outgrowth. To better understand the molecular mechanisms, we monitored global gene expression changes by DNA microarray analysis of 8,000 genes to study MeHg-regulated gene expression. Differentially expressed genes (10 up and 16 down-regulated genes compared of differentiated cell) were identified in differentiating cells. Clustering analysis revealed some novel changes in the expression of genes that appeared to be associated with differentiation of neuron, cytoskeleton, cell cycle, ion transport and cell signalling, etc. Finally, to better understand the molecular mechanisms, we assessed the effects of MeHg on cell cycle and related gene expression. SH-SY5Y cells were arrested in G1 phase after differentiation by RA. By 1.8μM MeHg treatment for 48 h, undifferentiated cells were arrested mostly in S phase, differentiating cells were arrested in S and G2/M phase and differentiated cells were observed a weak S phase arrest. Over-expression of p21 mRNA and inhibition of reduced phosphorylation of p53 protein by MeHg was observed in differentiated and differentiating cells. However a marked increase in phosphorylation of ERK1/2 was observed in only differentiating cells exposed to MeHg. Therefore, we suggested that human neuronal cell cycle (S and G2/M phase) arrest by exposure of MeHg during differentiation was associated with inhibition of cyclin A/B1-Cdk by activation of ERK1/2 through p53-independent pathway.

In summary, this thesis represents predictive signatures of exposure and effects of MeHg viewed from different angles using toxicogenomic tools like as the genome-wide analysis of gene expression. Time responsive effects and molecular target genes of MeHg in human neuronal cells and mechanistic results associated with neurotoxicity during differentiation phase were investigated. Through further investigation *in vitro* and *in vivo*, it should be characterized completely the neurotoxic mechanisms of MeHg and applied as biomarker for risk assessment of MeHg in human population.