독성 유전체 방법을 이용한 메칠수은의
신경독성에 대한 연구

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Toxicogenomic Solution on Neurotoxicity of Methylmercury

(I) Identification of Differentially Expressed Genes by Methylmercury in Neuroblastoma cell line using suppression subtractive hybridization (SSH) ; Methylmercury(MeHg), one of the heavy metal compounds, can cause severe damage to the central nervous system in humans. Many reports have shown that MeHg is poisonous to human body through contaminated foods and has released into the environment. Despite many studies on the pathogenesis of MeHg-induced central neuropathy, no useful mechanism of toxicity has been established so far. In this study, using of SSH method, was performed to identify d'fferentially expressed genes by MeHg in neuroblastoma cell line. We prepared to total RNA from SH-SY5Y (human neuroblastoma cell line) treated with solvent (DMSO) and 6.25 uM (IC50) MeHg. And we performed forward and reverse SSH on mRNA derived from SH-SY5Y treated with DMSO and MeHg (6.25 uM). Differentially expressed cDNA clones were sequenced and were screened by dot blot and real time RT-PCR to confirm that individual clones indeed represent differentially expressed genes. These sequences were identified by BLAST homology search to known genes or expressed sequence tags (ESTs). Analysis of these sequences may provide an insight into the biological effects of MeHg in the pathogenesis of neurodegenerative disease and a possibility to develop more efficient and exact monitoring system of heavy metals as ubiquitous environmental pollutants.

(II) Identification of Genes associated with Early and Late response of Methylmercury in Human Neuroblastoma Cell Line ; Methylmercury is known to have devastating effects on the mammalian nervous system. In order to characterize the mechanism of methylmercury-induced neurotoxicity, we investigated the analysis of transcriptional profiles on human 8k cDNA microarray by treatment of 1.4 uM methylmercury at 3, 12, 24 and 48hr in human neuroblastoma SH-SY5Y cell line. The methylmercury response of some of the identified genes was significant at early time points (3hr), that of others was at late time points (48hr). The early response genes that may represent those involved directly in the methylmercury response included pantothenate kinase 3, A kinase (PRKA) anchor protein (yotiao) 9, neurotrophic tyrosine kinase, receptor, type 2 gene, associated with NMDA receptor activity regulation or perturbations of central

nervous system homeostasis. Also, when SH-SY5Y cells were subjected to a longer exposure (48h), a relative increase was noted in a gene, glutamine-fructose -6-phosphate transaminase 1, reported that overexpression of this gene may lead to the increased resistance to MeHg. To confirm the alteration of these genes in cultured neurons, we then applied real time-RT PCR with SYBR green. Thus, this results suggest that a neurotoxic effect of the methylmercury might be ascribed that in the early phase, methylmercury alters NMDA receptor regulation or homeostasis of neuronal cells but in the late phase, it protects cells from effects of methylmercury.

(III) Selenoprotein W as Molecular Target at the Late Phase after Mehtylmercury Exposure in SH-SY5Y human neuroblastoma cell line ; From our previous transcriptional profiling study, we identified that selenoprotein W was down-regulated in SH-SY5Y neuroblastoma cells exposed 1.4 uM methylmercury (MeHg) for 12, 24, 48hr. To further investigate the effects of MeHg on selenoenzymes in the human neuroblastoma cells and especially, to understand the mechanism on down-regulation of selenoprotein W by MeHg, mRNA levels of selenoenzymes, selenoprotein P (SelP), W (SelW), glutathione peroxidase 4 (GPX4), 5-iodothyronine deiodinases (5-DI) and 5'-DI were evaluated using real time RT-PCR. In early phase, MeHg did not affect the level of SelW and 5-DI, while it significantly inhibited expression of SelP, but increased in the level of 5'-DI. And in the late phase, MeHg did not affect the level of SelP and 5'-DI, while it significantly inhibited expression of SelW. GPX4 decreased in the early and late phase. Especially, to understand the mechanism on down-regulation of selenoprotein W by MeHg, we suggest three hypothesis; (1) Se-Hg complex formation → SelW decreasing; (2) ROS generation by MeHg → inactivation of SelW; (3) GSH depletion by MeHg → decreasing of SelW, GSH-dependent antioxidant. Selenium had no effect on cytotoxicity of MeHg, down regulation of SelW by MeHg and formation of ROS by treatment with MeHg. Glutathione decreased on cytotoxicity by MeHg, repaired on down regulation of SelW by MeHg and weakly decreased on formation of ROS by treatment with MeHg. Also other heavy metal (Cu2+ & Fe2+) revealed the different pattern with MeHg on mRNA expression of Selenoprotein W. This results suggest that MeHg specifically altered the metabolism of intracellular GSH or Ca^{2+} and regulation of selenoenzymes in neuronal cells. Significance of the phase dependent alteration of the activities of selenoenzymes such as SelP, SelW, GPX4, DIs by MeHg exposure, especially sel W as molecular target in the late phase are discussed in relation to the neurobehavioral toxicity of MeHg.

(IV) Gene Expression Profiling of Methylmercury to Reveal Potential New Mechanistic Markers of Neurotoxicity in Nerve Differentiation Phase ; Methylmercury (MeHg) has been an environmental concern to public health and regulatory agencies for over 50 years because of its toxicity to the human nervous system. 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 (fetal model) and differentiated (adult model) SH-SY5Y neuronal cell models were applied in this study. First, the effects of MeHg on neurite outgrowth and cell viability were quantified at each cell models. In differentiating and differentiated cells, following 48-h exposure, 1.8 uM MeHg significantly decreased retinoic acid (RA)-stimulated neurite outgrowth. Cell viability was assessed in the same cultures by MTT assay. In undifferentiated cells, the IC_{20} of MeHg was I.8 uM. In differentiating and primed cells, the cytotoxicity of MeHg were at least 5-fold higher than undifferentiated cells. To better understand the molecular mechanisms, we monitored global gene expression changes by DNA microarray analysis of 8000 genes to study MeHg-regulated gene expression in the undifferentiated (no RA+MeHg), differentiating (RA+MeHg) and differentiated (MeHg after RA treatment) cells. This study detected a significant difference of gene expression profiles in nondifferentiated cells and RA-differentiating cells treated MeHg for 48hr. Up & down regulated genes identified through this study may be applied to elucidate mechanisms of MeHg neurotoxicity in developing (differentiating) neuronal system. 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. Among theses cellular responses, to elucidate mechanism related to decrease of RA-induced neurite outgrowth by MeHg we investigated to mRNA expression of cell adhesion molecules and Rho family of small GTPase (ras related). Cell surface proteins that play critical roles in cell recognition and cell adhesion, cadherins, integrins, actin, tubulin, and a variety of immunoglobulin-like molecules are essential for a wide variety of physiologic processes such as epithelial barrier function, tissue development, learning and memory, and immune responses. Down expression of N-cadherin and related genes by MeHg may be inhibit neurite outgrowth and path-finding in neural cell. Perturbation in transcriptional and/or posttranslational control of N-cadherin during development is associated with adverse effects on both brain morphology and neurobehavior (Bennett *et al., 1997; Dey et al., 2000).* Also, small GTPase RhoG, key regulator for neurite outgrowth in neuronal cells decreased and Rnd3 (RhoE) which inhibits the formation of actin stress fibers and the assembly of focal adhesions increased in transcriptional level. We conclude that gene expression profiling coupled with exposure of MeHg during differentiation affords promising opportunities to reveal potential new mechanistic markers of toxicity in developing neuron system.