### AS3-3

**Epigenetic dysregulation during chemical carcinogenesis**

Yongmei XIAO¹, Daochuan LI¹, Bo ZHANG¹, Qing WANG¹, Xiaowen ZENG¹, Ping YANG¹, Huawei DUAN², Zhixiong ZHUANG¹,³, Yuxin ZHENG², Wen CHEN¹

¹Department of Toxicology, School of Public Health, Sun Yat-sen University, China, ²Key Laboratory of Chemical Safety and Health; National Institute for Occupational Health and Poison Control, Chinese Center for Disease Control and Prevention, China, ³Shenzhen Center for Disease Control and Prevention, China

Analysis of the changes in different stage of human cell transformation may yield insights into the multiple events involved in acquisition of the tumorigenic phenotype induced by chemical carcinogens. In this study, we treated human cell lines with known carcinogens and obtained malignant phenotype of cell transformation confirmed by injection subcutaneously in immunodeficient mouse. We then screened differentially methylated genes or miRNAs at different stages of cell transformation using CpG island and miRNA microarray. As a result, we identified that 83 hypermethylated promoters were differentially modified in transformed cells. Among which 17 genes mRNA levels were decreased significantly and methylation at CpG islands of promoters. Particularly, we examined the methylation status of p16INK4α promoter in peripheral blood lymphocytes (PBLs) of 69 polycyclic aromatic hydrocarbons (PAHs) exposed workers. Among the 35 CpG sites we analyzed, 22 were highly hypermethylated in PAHs-exposed group. These 22 hypermethylated CpG sites were positively correlated to levels of urinary 1-hydroxypyrene (1-OHP) and the cytokinesis-block micronucleus (CBMN) in PBLs. In addition, miRNA expression profiles of BaP-transformed HBE cells revealed that 12 miRNAs differentially expressed at both pre-transformed and transformed stages. Among these miRNAs, down-regulation of miR-638 was found in 68% (34/50) of human NSCLC tissues. The average expression level of miR-638 in PBLs from 86 PAHs exposed workers increased by 72% compared with control group. Moreover, we demonstrate that miR-638 is involved in the BaP-induced carcinogenesis by targeting BRCA1. Taken together, our findings suggest that aberrant epigenetic regulation can be potentially used as biomarkers for risk assessment.

### AS3-4

**Keap1-Nrf2 system for maintenance of redox homeostasis**

Keiko TAGUCHI¹, Nanako FUJIKAWA¹, Hozumi MOTOHASHI², Masayuki YAMAMOTO¹

¹Department of Medical Biochemistry, Graduate School of Medicine, Tohoku University, Japan, ²Center for Radioisotope Sciences, Graduate School of Medicine, Tohoku University, Japan

The Keap1-Nrf2 system plays a central role in cytoprotection against oxidative and electrophilic insults. Under normal conditions Keap1 serves as a substrate adaptor for Cullin3-based ubiquitin E3 ligase and promotes proteasomal degradation of Nrf2. Keap1 is inactivated upon the exposure to environmental stimuli such as electrophiles, and in this situation Nrf2 is stabilized and activates transcription of cytoprotective genes. Nrf2 knockout mouse (Nrf2º/º) is very sensitive to toxicants like acetaminophen. On the other hand, Keap1 knockdown mouse (Keap1¹³⁰⁰/¹³⁰⁰) in which Nrf2 is activated is resistant to toxicants. Whereas the turnover of Nrf2 has been well analyzed, little is known about the Keap1 turnover or recovery of the Keap1 activity after the electrophilic insults. We found that Keap1 is accumulated when autophagy is impaired in the liver of mice. Treatment of cells with an autophagy inhibitor or inducer markedly increased or decreased the Keap1 level, respectively. The Keap1 degradation was accelerated upon the exposure to certain type of electrophiles, implying that inactivated Keap1 after modification with electrophiles becomes a preferred substrate of autophagy. We also found that the electrophilic challenge enhances the transcription of Keap1 gene, suggesting that the Keap1 activity is recovered by de novo synthesis of Keap1. Consequently, Keap1 protein was kept in constant level even in the presence of electrophiles. These results thus demonstrate that Keap1 is degraded through autophagy and that this degradation machinery in concert with the de novo synthesis of Keap1 maintains the active Keap1 level and the redox homeostasis regulated by Nrf2.