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Differential expression of cytochrome P450 genes associated with benzene-induced hematotoxicity

Zhiwei Zhao[1], Yongyi Bi[1], Ying Xia[1], Ning Tao[1], Qiang Ma[2]. [1]Occupational and Environmental Health, Wuhan University School of Public Health, 185 Donghu Rd., Wuhan, Hubei, 430071, China, People's Republic of, [2]Receptor Biology Laboratory, TMBB/HELD, National Institute for Occupational Safety and Health, CDC, 1095 Willowdale Rd., Morgantown, WV, 26505

Benzene is an established human carcinogen and leukemogen. Chronic benzene exposure results in progressive depression of bone marrow function with increased risks of aplastic anemia, myelodysplastic syndrome (MDS), acute myeloid leukemia (AML), chronic lymphocytic leukemia, and other disorders. The mechanism underlying benzene toxicity remains uncertain; an initial metabolism and bioactivation of benzene, especially by CYP2E1 in the liver, was considered a prerequisite. To identify CYP genes whose expression is aberrant in benzene poisoning, a cDNA microarray containing 32 CYP genes was used to detect differential expression of CYPs in patients with hematopoietic dysfunction of benzene exposure. Seven female shoemakers with hematological disorders (six cases of decreased peripheral white blood cells and one case of aplastic anemia) were recruited, and seven age- and gender-matched normal subjects were selected as controls. Total RNA from the two groups was prepared, followed by reverse transcription to cDNAs with concomitant incorporation of fluorescent dCTP (Cy3 for the patients and Cy5 for the controls). Microassay was performed. Genes with a two-fold higher/lower expression level were considered to be significant. Six CYP genes (CYP4F3, CYP1A1, CYP27A1, CYP1B1, CYP2B6, and CYP51) were found to be differentially expressed between benzene-exposed patients and controls. Among these, CYP4F3 gene was up-regulated consistently among all patients. Our study indicated that CYP4F3 is up-regulated in peripheral blood cells in bezene poisoning and may serve as a new biomarker for the exposure and poisoning of benzene.

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Protection against Chromium (VI)-induced oxidative stress and apoptosis by Nrf2. recruiting Nrf2 into the nucleus and disrupting the nuclear Nrf2/Keap1 association by toxic metal

Xiaoqing He, Gary X. Lin, Michael G. Chen, Qiang Ma. Receptor Biology Laboratory, Toxicology and Molecular Biology Branch, Health Effects Laboratory Div., National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, 1095 Willowdale Rd., Morgantown, WV, 26505

Chromium(VI) (Cr) is a major environmental toxic metal and human carcinogen. The molecular events mediating cellular responses to Cr(VI) are not clear at present. We show that Cr(VI) potently induced apoptosis and production of reactive oxygen species (ROS) in a concentration-dependent manner. Mouse embryonic fibroblast cells lacking Nrf2 exhibited elevated ROS production and apoptosis, which were markedly further increased by Cr(VI). Protection by Nrf2 correlated with induction of cytoprotective genes Ho-1 and Nqo1. Induction of the genes by Cr(VI) involved inhibition of ubiquitination of Nrf2 and accumulation of Nrf2 into the nucleus. In the nucleus, treatment with Cr(VI), but not phenolic antioxidant tBHQ, librates Nrf2 from Nrf2/Keap1 association and recruits Nrf2 to the antioxidant response elements (ARE) located in the enhancers of Ho-1 and Nqo1. Activation of Nrf2 by Cr(VI) was accompanied by the nuclear translocation and deubiquitination of Keap1 implicating recycling of Keap1 in Nrf2 signaling. Thus, protection against Cr(VI) toxicity involves a transcriptional signaling loop that includes activation of Nrf2 signaling by toxic metal, transcription of ARE-driven genes, and reduction of ROS production.

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Role of $G\alpha_{12}/G\alpha_{13}$ as novel switches for the activity of Nrf2

Won Dong Kim[1], Min Kyung Cho[1,2], Chang Ho Lee[3], Sang Geon Kim[1]. [1]College of Pharmacy, Seoul National University, San 56-1, Sillim-dong, Gwanak-gu, Seoul, 151-742, Korea, Republic of, [2]College of Oriental Medicine, Dongguk University, 707, Seokjang-dong, Kyungju, 780-714, Korea, Republic of, [3]Department of Pharmacology, College of Medicine, Hanyang University, 17 Haengdang-dong, Seongdong-gu, Seoul, 133-791, Korea, Republic of

$G\alpha_{12}$ family members are activated by various stimuli, leading to the regulation of physiological processes. Oxidative stress has damaging effects on cells and trigger defensive responses. The transcriptional activation of antioxidant genes depends on Nrf2 activity. This study investigated the regulation of Nrf2 by $G\alpha_{12}/G\alpha_{13}$. A deficiency of $G\alpha_{12}$, but not of $G\alpha_{13}$, enhanced Nrf2 activation and target gene transactivation. In mice, $G\alpha_{12}$ knockout activated Nrf2 and thereby facilitated heme catabolism to bilirubin and its glucuronyl conjugations. Microarray and real-time PCR experiments demonstrated that $G\alpha_{12}$ deficiency enhanced tert-butylhydroquinone induced Nrf2 target genes transactivation. The absence of $G\alpha_{12}$ or $G\alpha_{13}$ reduced the JNK-dependent ubiquitination of Nrf2. The absence of $G\alpha_{12}$, not $G\alpha_{13}$, increased PKCδ activation and the PKCδ-mediated serine phosphorylation of Nrf2. $G\alpha_{13}$ knockout or knockdown abrogated the Nrf2 phosphorylation induced by $G\alpha_{12}$ deficiency, indicating that relief from $G\alpha_{12}$ repression leads to the $G\alpha_{13}$-mediated Nrf2 activation. Overexpression of dominant negative Rho mutant abrogated the Nrf2 activation by $G\alpha_{13}QL$, thereby repressing its target gene. In summary, $G\alpha_{12}/G\alpha_{13}$ transmit a JNK-dependent signal for Nrf2 ubiquitination, whereas $G\alpha_{13}$ regulates Rho-PKCδ-mediated Nrf2 phosphorylation, which is negatively balanced by $G\alpha_{12}$.

886.6

Potential mechanisms for the decreased expression of the CYP2A13 7520C>G variant allele

Hong Wu, Xiuling Zhang, Guoyu Ling, Jaime D'Agostino, Xinxin Ding. Wadsworth Center, NYS Department of Health, School of Public Health, SUNY at Albany, Empire State Plaza, Albany, NY, 12201-0509

Our aim was to identify genetic polymorphisms and epigenetic factors that influence CYP2A13 expression. CYP2A13, expressed mainly in the respiratory tract, is highly efficient in the metabolic activation of tobacco-specific nitrosamines. Large interindividual differences exist in the expression of CYP2A13, which likely contribute to the differing susceptibility to lung cancer among smokers. A common 7520C>G variation in CYP2A13 was recently found to be associated with decreased allelic expression in human lung. In vitro data indicated that this SNP, located in the 3'-untranslated region, does not cause changes in the stability of the CYP2A13 transcript. Furthermore, the decreased CYP2A13 expression appeared to be manifest at the transcriptional level. Thus, we also explored possible roles of CYP2A13 promoter region DNA methylation in the decreased expression of the 7520G allele. An allele-specific CpG site, at −1479, which is associated with 7520G, was found to be methylated, for the most part, in human lung DNA samples. Gel shift assays detected specific binding of proteins to the −1479C variant probe, but not to the −1479T "wild-type" probe. Reporter gene assays demonstrated a small, but significant, decrease in CYP2A13 promoter activity associated with the -1479T>C variation. These findings provide the basis for further studies of the mechanisms of regulation of CYP2A13 expression, and for identification of genetic markers for lung adenocarcinoma susceptibility. (Supported in part by NIH grant CA092596)

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Repeated Administration of Oxycodone Alters the Expression of Drug Metabolizing Enzymes in the Liver of Sprague-Dawley Rats

Alan L. Myers, Hazem E. Hassan, Insong J. Lee, Natalie D. Eddington. Pharmacokinetics-Biopharmaceutics Laboratory, Department of Pharmaceutical Sciences, University of Maryland at Baltimore, 20 Penn Street, Baltimore, MD, 21201

Oxycodone is an opioid receptor agonist commonly prescribed to treat moderate to severe pain. Our objective was to study the effect of repeated administration of oxycodone to rats on the expression of genes encoding for drug metabolizing enzymes in the rat liver. Briefly, Sprague-Dawley rats were administered oxycodone (15 mg/kg) or saline i.p. twice daily for 8 days. Rats were sacrificed and RNA was isolated

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