1466 BaP Exposure Increases LINE-1 ORF1 Protein Export in Lung Epithelial Cell Exosomes

A. M. Cavalcante, E. C. Bowers, and K. S. Ramos. *University of Arizona, Tucson, AZ.*

Exosomes are vesicles of endocytic origin that can induce physiological changes in recipient cells. As such, exosomes may have an undiscovered role in mediating the effects of chemical exposures in tissues that significantly influence health outcomes. There is compelling evidence connecting cigarette smoking and lung cancer; however, the mechanisms and novel biomarkers are still being defined. We hypothesize that Long Interspersed Nuclear Element 1 (LINE-1) levels inside lung epithelial cell exosomes serve as a biomarker and mediator of smoking-related lung carcinogenesis. LINE-1 is a retrotransposon that can replicate and insert itself into different loci through reverse transcription. LINE-1 is epigenetically silenced in healthy cells and activated by harmful environmental exposures such as tobacco smoke. Furthermore, LINE-1 activation stimulates oncogenic phenotypes in lung epithelial cells. To investigate this hypothesis, we treated lung epithelial cells for 48 hours with different concentrations of benzo(a)pyrene (BaP), a cigarette smoke carcinogen known to induce LINE-1, and quantified levels of LINE-1 protein (ORF1) in cells and exosomes by Western blotting. We found that BaP increased ORF1 levels up to 2.3-fold in treated cells compared to vehicle controls. We discovered that ORF1 protein was secreted inside exosomes and that ORF1 protein exosome levels reflected cellular levels. These data support the hypothesis that LINE-1 protein levels in exosomes serve as a biomarker of environmental exposures associated with lung cancer.

1467 Nephrotoxic Biomarkers in Gentamicin-Induced Acute Kidney Injury for Hazard Identification

S. Lee[1,2], S. Hwang[1,2], N. Tham[1], J. Bang[1], H. Yi[1], B. Jeon[1], H. Lee[3], G. Woo[2], H. Kang[1], Y. Kim[1], and H. Ku[1]. *[1]Toxicological Evaluation Laboratory, Animal and Plant Quarantine Agency, Gimcheon, Korea, Republic of; [2]Semyung University, Jecheon, Korea, Republic of; and [3]Animal Pathodiagnostic Laboratory, Animal and Plant Quarantine Agency, Gimcheon, Korea, Republic of.* Sponsor: Y. Chung

Biofluids-based microRNAs (miRNAs) have been explored as biomarkers for predicting toxicity of chemicals. Here, we evaluated that miRNA biomarkers could be employed as nephrotoxic endpoints in *in vivo* acute kidney injury models. For this, the SD rats were subcutaneously administered daily with gentamicin sulfate (single dose of 400 or 600 mg/kg, and repeated doses of 200 or 600 mg/kg for 3 days). Acute kidney injury was confirmed by clinical endpoints (the elevation of blood urea nitrogen, serum creatinine and urinary Kidney injury molecule-1, or the presence of histopathologic lesion). About 50 genes were selected as literature-based candidate miRNA biomarkers by reviewing previous studies. Among these genes, miR-378a-3p was significantly upregulated in serum and urine of rats administered to repeated doses of gentamicin sulfate (600mg/kg). In addition, miR-26b-5p, miR-34a-5p, miR-320-5p and miR-345-3p were increased with more than 3-fold changes in rat urine of same treatment groups. These findings suggested that miRNA biomarkers may be useful as additional endpoints for predicting nephrotoxic potential of chemicals.

1468 Assessment of Welding Fume Exposure on Telomere Length and Regulation in Peripheral Blood Mononuclear Cells and Lung Tissue in Rats

M. Shoeb, V. Kodali, T. Meighan, K. Roach, X. Xin, G. Boyce, J. Roberts, A. Erdely, and J. Antonini. *NIOSH, Morgantown, WV.*

Telomeres are DNA fragments at the ends of chromosomes that protect genetic information during cell proliferation. Telomeres control DNA damage response (DDR) and DNA repair activity during cell division by regulating ATM and ATR kinases. Protection of telomeres 1 (POT1) protein specifically binds the 3' overhang of the telomere and plays a key role in chromosomal end protection and telomere length regulation. In this study, we examined POT1 mRNA expression and telomere length and regulation by shelterin complex proteins in peripheral blood mononuclear cells (PBMCs) and non-lavaged whole lung tissue in male Sprague-Dawley rats following exposure, by intratracheal instillation, to 2 mg/rat of manual metal arc-stainless steel welding fume (WF) particulate or saline (vehicle control). PBMCs and lung tissue were harvested at 30 d after instillation. WF is a complex mixture of metals (41% Fe, 28% Cr, 17% Mn, 3% Ni) with a count mean diameter of 600 nm. The PBMCs recovered from WF-exposed animals had increased telomere length as analyzed by fluorescent *in situ* hybridization (FISH), flow cytometry, and qPCR compared to controls. Altered expression of shelterin regulatory proteins, tripeptidyl-peptidase 1 (TPP1) and TERF1-interacting nuclear factor 2 (Tin2), was observed in PBMCs and lung tissues. Also, increased telomere length in lung tissue as analyzed by qPCR was observed in the WF group compared to control. However, qPCR analysis showed that POT1 expression levels in the lung tissue of the WF group relative to those in the control group (T/N ratio) was significantly lower, leading to the activation of ATR expression that was not observed in PBMCs. These results indicate that exposure to WF down-regulated lungs POT1 which in turn activated ATR-dependent DNA damage signaling and telomere elongation in the lungs, as well as activation of telomerase-independent pathway, an alternative mechanism leading to telomere elongation in PBMCs.

1469 miRNA Profile Assessment of Urine Exosomes from Boric Acid Treated Rats as Potential Biomarkers for Testicular Toxicity

R. W. Ball[1], B. R. Gould[2], and J. Wise[2]. *[1]REACH Borates Consortium, Brussels, Belgium; and [2]QIAGEN Inc., Germantown, MD.*

The objective of this study is to identify miRNAs in urine specific to boric acid (BA) related reproductive effects in rats. Urine samples were evaluated to identify differentially expressed testicular-specific miRNAs in BA treated rats using next-generation sequencing (NGS). Boric acid was administered to male Sprague Dawley rats via oral gavage at a dose level of 500 mg BA/kg bw/day for 28 days, a dose level known to produce fertility effects in male rats with minimal overt signs of toxicity. At the end of 28-days urine was collected over 24 hours. Urine samples were shipped to QIAGEN Genomic Services for exosomes isolation and miRNA analysis. Histopathology was conducted on testes and epididymis confirming BA treatment related effects. Test substance-related findings included lower epididymis weights, smaller epididymides, and microscopic findings of cellular debris and decreased spermatid cellularity in the epididymis and tubular degeneration/atrophy and atypical residual bodies in the testis. Tubular degeneration/atrophy in the test substance-treated group was characterized by decreased numbers of germ cells with degeneration of spermatocytes and spermatids. Urine exosomes and miRNA were isolated with QIAseq 52 Spike-ins through exoRNeasy. miRNA sequencing was performed using an Illumina NextSeq500. The miRNA library preparation was completed using the QIAseq miRNA Library Kit. Several miRNAs were identified in rat urine as possible biomarkers for BA related effects on male reproductive system: miR-34c-5p, miR-449a-5p and miR-122-5p were decreased in BA treated rats compared to untreated controls. These miRNAs have also been identified to be decreased in humans with sertoli cell related spermatogenic failure. BA has been shown to affect Sertoli Cells in the testes of rats. Additionally, let-7-5p (decreased), mir-141-3p (increased) and miR21-5p (increased) were differentially expressed in BA treated rats. These miRNAs have been identified as potential biomarkers for human male non-obstructive azoospermia. Also, miR-27b-3p levels decreased in BA treated rats shown to be associated with asthenozoospermia in humans. These results provide the basis for the potential application of miRNAs as biomarkers for BA related testicular toxicity and highlight the value of urine exosome miRNA NGS as a discovery tool.

1470 Transient Alanine Aminotransferase Increases following Acetaminophen Treatment in Rats

W. B. Mattes[1], A. Regev[2], R. J. Church[3], P. B. Watkins[3], M. Avigan[4], D. L. Mendrick[1], J. J. Greenhaw[1], and Q. Shi[1]. *[1]US FDA/NCTR, Jefferson, AR; [2]Eli Lilly and Company, Indianapolis, IN; [3]Institute for Drug Safety Sciences, Research Triangle Park, NC; and [4]US FDA/CDER, Silver Spring, MD.*

A persistent concern in pharmaceutical development is the appropriate response to serum alanine aminotransferase (ALT) increases seen in patients given a new drug in clinical trials. While ALT increases can portend serious liver injury, it is also well known that for certain drugs, such increases in clinical settings reverse with continued treatment, with no further evidence of liver injury. This well-documented phenomenon is often referred to as adaptation. A means to distinguish those ALT increases that will resolve from those that portend serious liver injury is needed. We report here a pilot animal model of such transient ALT increases. Sprague-Dawley rats were given daily oral doses of 1.0, 1.5 and 2.0 g/kg acetaminophen (APAP) and sacrificed after 8 days of treatment. Tail bleeds were conducted at days 1, 3, 6 and 7 of treatment. ALT increases were seen at day 3 in APAP treated rats. Strikingly, 1) these increases were not seen in all treated animals and the extent varied greatly from animal to animal; 2) all ALT increases returned to baseline by day 6; 3) the increases were not dose dependent; 4) by contrast, no increases in total bilirubin were seen; 5) while minimal to mild centrilobular necrosis was observed at day 8

58TH ANNUAL MEETING & ToxExpo · MARCH 10–14, 2019

The Toxicologist

Supplement to *Toxicological Sciences*

OXFORD
UNIVERSITY PRESS

ISSN 1096-6080
Volume 168, Issue 1
March 2019

www.academic.oup.com/toxsci

The Official Journal of the Society of Toxicology

SOT | Society of Toxicology

Creating a Safer and Healthier World by Advancing the Science and Increasing the Impact of Toxicology

www.toxicology.org

Publication Date: February 18, 2019