## 3034 A Cell-Based Model for Studying Human Angiotensinogen Gene Regulation after Cytokines

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Human angiotensinogen (hAGT) is an acute phase response II (APR II) protein secreted from liver which plays a significant role in blood pressure regulation and mediating fibrogenic transformation of liver after hepatic injury. Since hepatotoxicants' exposure induce immune response after injury, the effects of cytokines on secretion of hAGT by HepG2 and Huh7 cells were studied after interleukins-1beta and interleukin-6 (IL-1β, and IL-6) and phorbol 12-myristate 13-acetate ester (PMA) treatments for 24 and 48 hrs. IL-6 treatment caused the maximum effects on hAGT secretion from HepG2 and Huh7 cells while IL-1β mediated hAGT activation were not comparable to IL-6. HepG2 cells were more responsive to hAGT activation by IL-6 as compare to Huh7 cells. The analysis of cellular level of AGT indicated that activation by IL-6, increased the cellular AGT levels in both HepG2 and Huh7 cells while treatments with IL-1β or PMA did not result into corresponding cellular increase. Differentially posttranslated cellular AGT forms were found with HepG2 and Huh7 cells after cytokines and PMA treatments. A gene reporter assay utilizing 2XNF-KappaB luciferase assay for confirming NF-κB mediated activation after treatment with IL-1β or PMA in both cell lines for either 24 or 48 hrs. was not very strong as anticipated. Although, NF-κB response site(s) has been demonstrated by Kobori et al in hAGT, it is concluded that hAGT gene activation in hepatocytes occurs predominantly by IL-6 and not by IL-1β.

## 3035 Thrombocytopenia Caused by panHDAC Inhibitors Correlates with Reduced GATA-1 Expression and Impaired Megakaryocyte Differentiation

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The effectiveness of HDAC inhibitors (HDACi), specifically panHDACi, against a broad range of cancers has been established in the clinic trials. Currently four HDACi (SAHA/Vorinostat, Panobinostat/LBH589, Romidepsin, and Belinostat/PDX101) have been approved by US FDA as epigenetic therapeutics, mostly for treatment of T cell lymphomas. Clinical trials for potential treatment of solid tumors require increased dosing; however, with potency comes thrombocytopenia, a common, dose-limiting adverse effect with an unclear etiology. Previous studies indicate potential involvement of GATA-1 and tubulin acetylation. We induced hematopoetic progenitor (CD34+) cells to differentiate *in vitro* for 14-days. The presence of panHDACi Panobinostat and SAHA at their respective clinical Cmax resulted in a significant reduction in MK differentiation. To dissect the underlying molecular mechanisms, we treated Meg-01 (a megakaryoblast line) with panHDACi for 2 days, and Western blotting analysis showed a total loss of GATA-1 accompanied by a robust increase in tubulin acetylation. In contrast, specific inhibition of HDAC5/4 with LMK235 had no effect on either GATA-1 protein levels or on MK differentiation. To further rule out a role of tubulin acetylation in MK differentiation, we treated Meg-01 cells with tubacin, an HDAC6 specific inhibitor, to induce tubulin acetylation. We observed that tubacin induced robust acetylation of α-tubulin but maintained GATA-1 levels generally unchanged. Finally, in the presence of tubacin, CD34+ cells remain the ability to successfully differentiate to MK. Taken together, our data indicate that panHDACIs cause thrombocytopenia by suppressing GATA-1 expression, thus blocking MK differentiation. Therefore, using GATA-1 levels as an indicator, identifying and developing new HDACIs that do not impair GATA-1 levels may avoid the dose-limiting thrombocytopenia associated with most panHDACis.

## 3036 The Molecular Mechanism of CSE Disturb Fibronectin Remold in the Vascular Endothelial Cells

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Smoking is harmful for the cardiovascular system, and is considered to be a major cause for atherosclerosis. Some chemicals in the cigarette can disrupt the microenviroment, which the vascular endothelial cells (VEC) reside in, inducing the disrepair of endothelial cells as well as a serial pathological responses, resulting in the arteriosclerotic vascular disease. However, the underlying mechanism for the alteration of VEC extracellular matrix, and when atherosclerosis forms, have not been discovered. In our preliminary experiment, we utilized a zebrafish model and tested the effect of the cigarette smoke extracts (CSE) on the vascular endothelial cells. We found that CES could induce abnormal deposition and aberrant assembly of Fibronection (FN) in the VEC. Meanwhile, the pivot genes, which regulate FN expression and assembly, such as snail, integrin, were changed as well. Based on these finding, we propose to further investigate the mechanism how the CES affect the assembly in the extracellular matrix by employing the zebrafish model and the human vascular endothelial cell lines. We will carry out the *in vivo* time course imaging, *in vitro* 3D culture to determine the CSE effects on the reconstruction of Fibronectin in VEC extracellular matrix during the atherosclerosis, and key factors involving in this process. Those studies of the regulatory mechanism and related pathways could help to better understand the pathogenesis of CSE-induced arteriosclerotic vascular disease and provide a potential avenue for prevention and treatment.

## 3037 3D Printer Emission Inhalation Stimulates Acute Hypertension and Microvascular Dysfunction

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Desktop 3D printers are now commonplace not only in research laboratories and industrial shops, but also offices and homes. We and others have reported that typical 3D printing with common acrylonitrile butadiene styrene (ABS) filaments generates high and mixed concentrations of ultrafine particulate matter (PM) and vapor emissions (3DPE). Despite this, containment strategies are lacking and the health effects of 3DPE inhalation are unknown. Because pulmonary exposure to a variety of airborne toxicants ranging from solid PM to gaseous components induces cardiovascular dysfunction, we hypothesized that 3DPE inhalation would lead to similar outcomes. Sprague-Dawley rats (53±2 days, 271±12 g) were exposed to 3DPE (N=7) or filtered air (sham, N=5) via nose-only inhalation exposures (DSI Buxco Inhalation Tower). 3DPE were generated by a Replicator 2x (Makerbot Industries) printing with black ABS, collected in a containment chamber and pumped into the Inhalation Tower. Exposure parameters: duration: 3-3.5 hrs; concentration: 0.9±0.1 mg/m$^3$; aerodynamic diameter 70±2 nm; mobility diameter 79±9 nm; and calculated lung deposition: 4.0±0.4 μg. 24 hrs post exposure, the spinotrapezius muscle was prepared for intravital microscopy and microvascular function was assessed via microiontophoresis (1-3 1$^{st}$ order arterioles studied/rat). Arterial pressure was directly measured in the carotid artery. At the time of intravital experiments, the 3DPE group displayed acute hypertension (mean pressure = 127±7 mm Hg) vs the sham group (89±5 mm Hg). Consistent with this elevated arterial pressure, arteriolar tone was significantly elevated (64±2% vs 55±5%, 3DPE vs sham, respectively). Further, endothelium-dependent arteriolar dilation (acetylcholine) was significantly attenuated in the 3DPE group (21±11% of maximum response) as compared to the sham group (70±9%). These results provide initial evidence that modest pulmonary 3DPE exposures may result in an acute systemic pressor response, characterized by an increased microvascular resistance and blunted dilator response. *The findings and conclusions in this report are those of the authors and do not necessarily represent the views of NIOSH. Mention of any company name or product does not constitute endorsement by NIOSH.* *Support: R01-ES015022 (TRN); NIOSH-NORA 63392 (TRN), 927ZLDM (ABS), and 63382 (JY).*

## 3038 MuRF1 Protects against the Functional and Metabolic Consequences of a Congenital Heart Defect That May Increase Susceptibility to Cardiovascular Toxins

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Toxins and environmental factors increase the incidence and severity of cardiovascular disease. Particulates, hormonal and environmental signals can activate nuclear receptors (NRs), a class of transcription factors that induce adaptive transcriptional responses. Multiple NRs such as glucocorticoid receptor (GR), thyroid hormone receptor alpha1 (TRα) and peroxisome proliferator-activated receptor alpha (PPARα) have altered activities in cardiovascular disease that contribute to the remodeling process that occurs during the development of heart failure. We have recently described a novel mechanism of regulation of nuclear receptors by the ubiquitin ligase MuRF1. Both TRα and PPARα are monoubiquitinated and translocated out of the nucleus by MuRF1, thereby reducing their transcriptional activity. In this study we combine a MuRF1-/- mouse model with a model of cardiac proteotoxicity, specifically the transgenic expression of a mutation in Myosin binding protein-C (MyBP-C) that causes a truncation known as m7t, to further investigate the mechanism of MuRF1 and its downstream targets in cardioprotection. By assessing cardiac function by conscious echocardiog-


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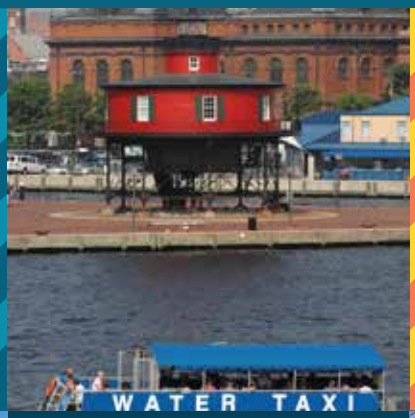


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