We stained the organoids with the following differentiation and intestinal region-specific antibodies by immunohistochemistry: zonula occludens-1 (ZO-1) for tight junction formation, mucin 2 for goblet cells, chromogranin A for enteroendocrine cells, and villin for enterocytes. Relative expression of lgr5 (adult stem cell marker), sucrose isomaltase (SI), villin and cholecystokinin (adult intestinal tissue markers) was measured by RT-PCR. As expected, lgr5 levels were down-regulated as SI, villin and cholecystokinin levels were up-regulated through the organoid differentiation period. Additionally, we used probe substrates for typical phase 1 and phase 2 drug metabolizing enzymes to assess organoid metabolism ability. Significant differences on metabolism between undifferentiated and differentiated organoids were observed. We aim to develop organoid structures from different segments of the intestine from human and preclinical species to provide a platform to investigate drug absorption and metabolism, intestinal physiology and disease, host-pathogen interactions and other toxicological questions.

1348 Discrepant *In Vitro* Cytotoxicity Results Can Be Explained by Method-Specific Differential Dependency on Pentose Phosphate Pathway-Associated Metabolism

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Tetrazolium reduction and resazurin assays remain the mainstay of routine *in vitro* toxicity batteries. However, neglecting to verify the baseline assumption of interaction of test article with method employed can produce potentially erroneous characterization of cytotoxicity/cell proliferation for test articles. The current investigation aimed to demonstrate the results of several standard cytotoxicity and proliferation assays varies in dependence on contributions from the pentose phosphate pathway (PPP). Beas-2B cells were treated with graded concentrations of benzo[a]pyrene (B[α]P) for 24 and 48 hours prior to cytotoxicity/proliferation assessment with commonly used MTT, MTS, WST-1, and Alamar Blue assays. B[α]P caused increased transient enhancement of metabolism of each dye assessed. An inhibitor of the glucose-6-phosphate dehydrogenase, 6-aminonicotinamide (6-AN), dose-dependently reduced metabolism of each endpoint listed in order of magnitude: WST-1 > MTS > MTT > Alamar Blue without overt cytotoxicity, implicating differential sensitivity of each endpoint to the pentose phosphate pathway (PPP). Nanomolar doses of B[α]P caused increases in all endpoints to suggest an increased cell proliferation. However, B[α]P treatment increased phosphorylation of Chk-1(S345) and p-53(S15), suggesting reductions in proliferative capacity. Reduction in proliferation was confirmed upon assessment of cell doubling time, which was lengthened dose-dependently. These results demonstrate differential sensitivity of studied cytotoxicity assessments on the PPP, thus 1) decoupling "mitochondrial activity" as an interpretation of cellular formazan and Alamar Blue metabolism, and 2) demonstrating the implicit requirement for investigators to sufficiently confirm methods of cytotoxicity/proliferation in routine experimentation. The nuances of these method-specific extramitochondrial metabolism must be scrutinized to properly qualify specific endpoints employed.

1349 Novel ID3 Regulatory Gene Networks Contributing to Brain Vascular Disease

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Cerebrovascular disease (CBVD) often leads to cognitive impairment and is a prominent comorbidity of Alzheimer's disease (AD) observed in 60%-90% of patients. Single nucleotide polymorphism in the human ID3 gene has been associated with atherosclerosis, however, its contribution to CBVD is unknown. This study uses a machine learning-based analysis of human brain microvessel transcriptome to identify novel ID3 gene regulatory networks in AD patients. Microarray data from human brain microvessels from 20 AD patients and controls were processed for normality. Probe set identifiers were mapped to Entrez identifiers, official gene symbols and gene names. Signal intensity values for each gene with multiple probes were averaged resulting in 21,000 unique gene annotations. Differential gene expression between case and controls was calculated by the Benjamin-Hochberg t-test ($p < 0.05$). ChIP-Seq analysis identified approximately 2,834 candidate genes bound by ID3 in endothelial cells. We discovered 38 ID3 bound target genes among 417 genes showing a 2-fold change between disease and non-disease brain microvessels. For Bayesian networks analysis, ID3 target genes were discretized in cases and controls. Variables such as age, gender, and disease were included into the Bayesian networks structure learning algorithm. Nine best structures were identified from running the learning algorithm nine times with different running times (three independent runs for 2 h, 4 h, and 8 h.). A Bayesian network structure identified at 8h was selected as the best scoring network comparing Bayesian Dirichlet scoring metric of all structures. The best scoring network showed a structural ID3 gene regulatory network interacting with age, gender, and disease. We discovered five key ID3 gene targets to be most influential to diseased brain microvessels (AMFR, BEX1, PARVG, PGM2L1, PRKACB). Furthermore, the best scoring network predicted a subject will have AD with a high probability (>0.999) when only three (BEX1, PGM2L1, PRKACB) of the five key genes are downregulated, and with a low probability (<0.001) when only PARVG was downregulated in human brain microvessels. Receiver Operating Curve analysis on ID3 gene network influence on disease resulted in an area under the curve of 0.75. Data driven machine learning is a powerful approach to predict ID3 causal gene networks involved in vascular disease. Given paucity of studies on human brain microvessels and small number of subjects in this study, a future goal will be to apply this method on a larger population with the hope of identifying blood-based biomarkers of vascular dementia.

1350 Assessment of Remediation of Arsenic from Water through Measuring Environmental Stressor Transcriptomic Gene Signatures

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Nuclear respiratory factor 1 (*nrf1*) is an environmental stressor gene, when exposed acutely by numerous environmental agents, such as temperature, physical activity, diet and accumulated metals it activates and regulates cellular functions. Molecular indices to measure Arsenic (As) in surface waters are currently unavailable. Here, we use the zebrafish which is a known sentinel for biomonitoring of aquatic environments. The objective in this study was to investigate *nrf1* transcription factor and its signature genes to identify orthologue signature gene networks which may be involved in the initiation and progression of cellular processes. To identify the common *nrf1* targets, we analyzed the ChIP-seq dataset from ENCODE. We then used bioinformatics and functional genomics to determine (As- *nrf1*) significant target genes comparing the treatment effects of low concentrations 15 (ppm) [As(V)] in liver hepatocytes while characterizing the cellular processes across 4 periods (8, 24, 48 and 96 h) of treatment time. The transcriptomic dataset of GSE3048 of 12 As treated and 12 control zebrafish samples was processed for normalization. A parametric *t* test, (*p*-value <0.05) between treated and control samples was used to determine differential expressed genes (DEGs) using the Benjamin-Hochberg False Discovery Rate (FDR). Dysregulated *nrf1* targets were determined based on a criterion of 2-fold change. During 8-96 h, we discovered 236 upregulated and 336 downregulated *nrf1* targets in As treated zebrafish. Furthermore, 8 h revealed 30 up and 72 down-regulated genes. A reduced number of downregulated genes were identified at 24 h, 60 up and 6 down-regulated genes. Relatively equal number up and down regulated genes occurred at 48 h with 57 up and 62 downregulated. Broadly, we found genes at 96 h most dysregulated compared to previous times including 89 up and 196 genes downregulated. Using the functional gene ontology (GO) and (KEGG) pathways using DAVID pipeline, revealed that 72.6 % of the upregulated and 69.7% of the downregulated *nrf1* target genes matched DAVID enrichment process. The most significant upregulated GO terms across 8-96 h included metabolic processing p value (2.3E-4), cellular processing *p* value (9.5E-3) and developmental processing p value (9.5E-2). Using KEGG, *nrf1* targets play a key role in cell cycle, RNA transport, p53 signaling and glycerophospholipid biosynthesis. Our results show that the altered transcriptome responds to arsenic treatment in a sensitive manner. This provides a molecular approach to supplement traditional analytical measurements detailing a comprehensive pathway framework to monitor As in surface waters.

1351 Toward Predictive Toxicology—Computable Biological Network Models of Drug-Induced Liver Injury

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Drug interactions with cigarette smoke impact the absorption, distribution, metabolism, and excretion of and, consequently, the response to some drugs. The main mechanism behind drug interactions with smoke involves hepatic metabolism, where polycyclic aromatic hydrocarbons present in cigarette smoke induce cytochrome P450 isoenzymes. The induction of these enzymes results in accelerated metabolism of xenobiotics in smokers and a clinically significant decrease in the pharmacologic effect. Xenobiotic metabolism pathways are also involved in drug-induced liver injury (DILI), where high-energy conversion of compounds can lead to necrosis or apotosis in the liver. To investigate how smoking affects DILI, we created a causal biological network model that gathered available biological knowledge into a structured and computable format. The resulting model provides a connected view of the signaling crosstalk that is critical for not only prediction of drug

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Preface

This issue is devoted to the abstracts of the presentations for the Continuing Education courses and Scientific Sessions of the 59th Annual Meeting of the Society of Toxicology, held at the Anaheim Convention Center, Anaheim, California, March 15–19, 2020.

An alphabetical Author Index, cross-referencing the corresponding abstract number(s), begins on page 542.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 580.

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