## 3102 Weighted Gene Coregulation Network Analysis (WGCNA) to Decipher Temporal Dynamics in Cardiotoxicity

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Cardiotoxicity can be elicited through a variety of mechanisms, which lead to complex, temporally regulated compensatory processes at the molecular level. The sequence of molecular processes has evolutionary roots that, at a regulatory level, are analogous to highly complex agent-based models with self-organized behavior. One approach to delineating the evolved, self-organizational framework is to characterize the coordinated expression genes across a diverse set of toxicological perturbations using WGCNA. Here we describe a cartographic exercise that uses WGCNA of the DrugMatrix heart gene expression data (1-5 days in duration, 88 test articles) from rat to reveal a map of gene-level co-expression relationships that are rooted in compensatory/adaptive processes associated with cardiotoxic stress. In total, the map reveals sets of genes tightly linked to well document biological processes such as cell cycle and ribosomal biogenesis, but also identifies sets of genes that are reflective of changes in cellularity and processes central to the differentiate functions of cardiac cells. We use the co-expression map as a base framework to explore the temporal dynamics of cardiotoxicity of a variety of prototype agents such as anthracyclines, corticosteroids, and kinase inhibitors. The exercise reveals early agent specific behavior that evolves into a general compensatory process that is likely intrinsic to the reparative function of the heart and is conserved across most test articles. We believe this analysis serves as molecular level point of reference that can be used to understand the capabilities and limitations of *in vitro* systems for modeling cardiotoxicity.

## 3103 A Targeted Metabolomics-Based Assay Using Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes Identifies Structural and Functional Cardiotoxicity Potential

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Implementing screening assays that identify functional and structural cardiotoxicity earlier in the drug development pipeline has the potential to improve safety and the cost and time required to bring new drugs to market. In this study, a metabolic biomarker-based assay was developed that predicts the cardiotoxicity potential of a drug based on changes in the metabolism and viability of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM). Assay development and testing was conducted in two phases: (1) biomarker identification and (2) targeted assay development. In the first phase, metabolomic data from hiPSC-CM spent media following exposure to 66 drugs was used to identify biomarkers that identified both functional and structural cardiotoxicants. Four metabolites that represent different metabolic pathways (arachidonic acid, lactic acid, 2'-deoxycytidine, and thymidine) were identified as indicators of cardiotoxicity. In phase two, a targeted, exposure-based biomarker assay was developed that measured these metabolites and hiPSC-CM viability across an eight-point concentration curve. Metabolite-specific predictive thresholds for identifying the cardiotoxicity potential of a drug were established and optimized for balanced accuracy or sensitivity. When predictive thresholds were optimized for balanced accuracy, the assay predicted the cardiotoxicity potential of 81 drugs with 86% balanced accuracy, 83% sensitivity, and 90% specificity. Alternatively, optimizing the thresholds for sensitivity yields a balanced accuracy of 85%, 90% sensitivity, and 79% specificity. This new hiPSC-CM-based assay provides a paradigm that can identify structural and functional cardiotoxic drugs that could be used in conjunction with other endpoints to provide a more comprehensive evaluation of a drug's cardiotoxicity potential.

## 3104 The Effects of Inhaled Multiwalled Carbon Nanotubes on Systemic Blood Pressure and the Autonomic Nervous System in Spontaneously Hypertensive Rats

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It is well documented that the autonomic nervous system (ANS) plays a critical role in controlling cardiovascular functions including heart rate, cardiac contractility and blood pressure (BP). In previous studies, we reported that inhalation of multi-walled carbon nanotubes (MWCNTs) elevated systemic BP, which was associated with an altered activity in the ANS in normotensive rats. In addition, we also reported that the BP of spontaneously hypertensive (SH) rats was greatly increased in response to pulmonary MWCNT exposure compared to normotensive rats. The available evidence indicates that in essential hypertension, there is a deteriorated balance between sympathetic and parasympathetic activity. The present study investigated the effects of inhaled MWCNTs on the ANS and the role of peripheral neurons in regulation of cardiovascular function after exposure of SH rats to MWCNTs. SH Wistar rats were pre-implanted with a telemetry device and exposed by inhalation to MWCNTs at a concentration of 2 mg/m$^3$ for 5 h/day for three consecutive days. The real-time EKGs and systemic BP were recorded by a telemetry system at pre-exposure, during exposure, 1 day post-exposure and 7 days post-exposure. The activity of the ANS in response to MWCNT exposure was determined by heart rate variability (HRV) analysis. The non-selective transient receptor potential (TRP) channel blocker, ruthenium red (2.5 mg/kg), was injected intraperitoneally 1 h before MWCNT exposure to study the role of peripheral neurons in regulating cardiovascular function and activity of the ANS after pulmonary MWCNT exposure. Inhalation of MWCNTs elevated systemic BP and increased the variance of the root mean square of successive differences (RMSSD) between adjacent R-R intervals ($p < 0.01$) and the high frequency (HF) power ($p < 0.01$). Both RMSSD and HF are metrics corresponding to autonomic nerve influences on cardiovascular function. Pretreatment with ruthenium red prevented those increases. Our study indicates that pulmonary exposure to MWCNTs significantly altered the activity of the ANS and increased the BP via a peripheral neuron-regulated pathway in SH rats.

## 3105 Cerebrovascular Dysfunction and Microvessel Density Changes in Offspring of Rat Dams Exposed to Electronic Cigarette Aerosols

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Electronic cigarette (E-cig) usage has steadily been increasing and it is even being promoted as a safer option to traditional tobacco cigarettes; however, there is little evidence supporting this theory as it relates to the cerebrovasculature. We examined the effects of maternal E-cig exposure (Joyetech eGrip OLED using 5-sec puffs @17.5 W) on cerebrovascular function and microvessel density in offspring with maternal exposure to ambient air (control, n=6), E-cig with 18 mg/ml nicotine (E-cig18, n=6), and without nicotine (E-cig0, n=7). Exposure consisted of 60 puffs over 1-hour each day, 5 days/week, and resulted in an average daily TPM of ~120 mg/m$^3$. Maternal exposure was started on gestational day 2 and continued until pups were weaned. Pups themselves we never directly exposed. The middle cerebral arteries (MCA) were obtained from 3-month old pups, isolated and positioned in a pressurized myobath, and exposed to increasing concentrations of acetylcholine (Ach; 10 -9 M to 10 -4 M), serotonin (5-HT; 10 -9 M to 10 -4 M), and sodium nitroprusside (SNP; 10 -9 M to 10 -4 M), in the presence or absence of Tempol (a superoxide dismutase mimetic). Brains were also flash frozen, sectioned, and analyzed for microvessel density (MVD). The MCA dilation of offspring to Ach was impaired in both E-cig0 and E-cig18 by 63% and 62%, respectively, compared to controls (<0.05). Incubation with tempol reversed the cerebrovascular dysfunction seen in both E-cig groups, suggesting the superoxide pathway is involved in the impairment observed in offspring with maternal E-cig use. The MCA dilation to SNP and constriction to serotonin was similar between all groups. Preliminary data (n=2 per group) shows a 12% and 29% decrease in MVD in the cortex of E-cig0 pups and E-cig18 pups, respectively. These data suggest that E-cig usage during pregnancy impairs the cerebrovascular reactivity and induces rarefaction of cortical microvessels in offspring.

## 3106 Developing Patient-Centric *In Vitro* Cardiovascular Safety Models Applicable to Drug Discovery

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Translation of functional and structural toxicity findings from healthy to disease states is a major challenge to providing safe and efficacious drugs. Currently, a range of *in vitro* and *in vivo* model systems are utilized to risk assess cardiovascular safety. Largely, these approaches rely on cells derived from 1-2 healthy donors or healthy animals that do not recapitulate patient's co-morbidities, pathophysiology of disease, drug treatment history or genetic variability. Critical to cardiomyocyte function is calcium signalling for which genetic variability within key calcium handling genes in the population exists. We hypothesized that hiPSC-CMs containing SNPs or reduced expression levels of a critical calcium handling gene, *RYR2* would display differential pharmacological responses compared to isogenic controls. This model would

SOT 2020

59th Annual Meeting & ToxExpo

Anaheim, California • March 15–19

# The Toxicologist

## Supplement to *Toxicological Sciences*


Toxicological Sciences

ISSN 1096-6080
Volume 174, Issue 1
March 2020

The Official Journal of the Society of Toxicology

OXFORD UNIVERSITY PRESS

SOT | Society of Toxicology

www.academic.oup.com/toxsci

Publication Date: February 21, 2020

# 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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To cite a 2020 SOT Annual Meeting abstract, please format as follows: *The Toxicologist*, Supplement to *Toxicological Sciences*, 174 (1), abstract #__, 2020, Title, First Author.

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