SESSION ID: 124
PRESENTATION TYPE: Symposium
SECONDARY PRESENTATION TYPE: Workshop
IAT/ITS Designation:
CME Consideration:

TITLE: Opening the Black Box: Understanding the Molecular Mechanisms of Developmental Toxicity

SESSION DESCRIPTION:
The development of an organism from egg to adult is a complex series of interlocked events, which depends on precise coordination in time and space. When xenobiotic agents interfere with development, they can alter cellular growth and differentiation, leading to permanent changes in tissue/organ structure and function, i.e. developmental malformations. Over the past 40+ years, developmental toxicologists have strived to understand the mechanisms of action resulting in these lesions. With the advent of novel molecular tools, an enhanced understanding of developmental biology, and new model systems, great progress has recently been made in deciphering some of the fundamental drivers of altered development. In this session, we will explore recent findings in developmental toxicology that have begun to link teratogens with their potential mechanisms of action. The first speaker uses a zebrafish cardiovascular model to explore the role of the G-protein-coupled estrogen receptor on heart rate. In the second presentation, the speaker describes a novel mechanism for arsenic disruption in TGFbeta-Smad signal transduction during formation of heart valves and coronary vessels. The third speaker demonstrates how altered TGF-beta and FGF signaling impact the development of the forebrain. Using an *in vitro* murine limb bud culture system and classic histone deacetylase inhibitors, the fourth speaker reveals the role of protein acetylation in the action of some developmental toxicants. Finally, using thalidomide and its analogs, the fifth speaker presents compelling data on the mechanism of thalidomide action in phocomelia. These speakers, who come from a mix of academic and industry backgrounds, will demonstrate how understanding the mechanisms underlying developmental toxicology allows for the prediction of class effects, the discovery of subtle but important developmental changes, and the design of more informative *in vitro* and *in vivo* methods for detecting teratogenicity.

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ENDORSER 2: Mechanisms Specialty Section
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Session Role Order: 1
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Funding: No SOT Funding
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Session Role Order: 2

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Session Role Order: 3
Session Role: Presenter
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Presentation Title: Acute Estrogen Exposure Increases Heart Rate via a G Protein-Coupled Estrogen Receptor Mechanism in Zebrafish
Presentation Description: Embryonic blood flow is a critical determinant of hematopoetic stem cell development and formation and growth of the heart; thus the establishment of normal embryonic heart rate is thought to be essential for proper development and function of the heart. Whether estrogenic environmental endocrine disruptors modulate heart rate is not well understood. Here we demonstrate that exposure to exogenous estrogens causes an acute increase in heart rate in zebrafish embryos via a G protein-coupled estrogen receptor (GPER). Pharmacological inhibition of estradiol synthesis caused a reduction in heart rate that was rescued by: 1) exogenous estradiol, 2) a membrane-impermeable estradiol, and 3) a specific GPER agonist. Exposure to a GPER agonist alone increased heart rate, while co-administration of a GPER antagonist blocked the increase in heart rate. To probe this signaling pathway further, we generated homozygous GPER mutant embryos, which exhibited a lower basal heart rate compared to wildtype embryos, suggesting that endogenous estrogens increase heart rate. Consistent with this hypothesis, we identified endogenous estradiol in embryos using a new mass spectrometry assay we developed. Using histology and genetic rescue techniques, we are working to identify the cell type(s) in which estrogens act to increase heart rate acutely. We are also testing whether GPER mutants exhibit abnormal heart morphology and altered heart valve structure in embryos and abnormal heart function in adult animals. Thus, studying the developmental toxicity of estrogenic endocrine disrupting compounds revealed a new physiological mechanism by which environmental and endogenous estrogens modulate heart rate, with implications for embryonic heart development and adult heart function.

Session Role Order: 4
Session Role: Presenter
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Funding: No SOT Funding Needed
Presentation Title: Fetal Arsenic Exposure Disrupts TGFß2-SMAD Signaling and Developmental EMT
Presentation Description: There is limited understanding on the molecular basis of developmental toxicity by arsenic. We reveal that TGFß2 signaling is a target of arsenic and as such disrupts key developmental processes. TGFß2 is a key growth factor regulating epithelial to mesenchymal transition (EMT) in several tissue compartments during embryogenesis. TGFß2 triggers cardiac progenitor cells to transform into mesenchymal cells and give rise to the cellular components of coronary vessels as well as cells of the heart valves. TGFß signaling is dependent on a dynamic on and off switch in Smad activity. Chronic arsenic exposure disrupts Smad activation leading to deficits in TGFß2 mediated EMT. In addition, TGFß2 induced Smad2/3 nuclear shuffling is attenuated during short exposures to arsenic independent of Smad phosphorylation or nuclear importation. This depletion in nuclear Smad is restored by knocking-down Smad specific exportins, Exportin 4 or CRM1, suggesting that arsenic augments Smad2/3 nuclear exportation. Zinc supplementation reverses the disruption in Smad2/3 nuclear translocation by arsenic revealing a novel mechanism. This coincides with Zinc rescue of arsenic mediated deficits in cardiac EMT. Thus, transformation and invasive cell motility of EMT derived mesenchyme are protected from arsenic toxicity by zinc supplementation. These findings establish a novel mechanism for arsenic disruption in TGFß-Smad signal transduction during development.

Session Role Order: 5
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Presentation Title: TGFß and FGF Inhibition Elucidates Mechanisms Controlling the Formation of the Diencephalon
Presentation Description: Inappropriate activation of the TGF-beta and FGF signaling pathways occurs in many disease states, including fibrosis and cancer, so receptors for both pathways have been targets for pharmaceutical inhibition. However, both pathways also play central roles in the development of the brain, particularly for the dorsal habenulae. These bilateral brain nuclei play a central role in both anxiety modulation and addiction. The habenulae are divided into medial and lateral subnuclei with distinct populations of neurons. Allocation of neurons to the subnuclei is controlled by the timing of the neuron's birth, with early-born neurons preferentially contributing to the medial subnucleus and later-born neurons to the lateral subnucleus. TGF-beta signaling contributes to the timing of habenular neurogenesis, but must use an intermediate signal, as TGF-beta ligands and receptors are no longer expressed when neurons are being produced. We find that FGF signaling is the intermediate signal. Using small-molecule inhibitors and activators of FGF signaling, we show that habenular neurogenesis is altered in a dose-dependent manner. Further, we identify that FGF signaling regulates expression of the cell-cycle-dependent kinase inhibitor (CDKI) kip2, providing a mechanism for FGF to control cell cycle exit and promote neuronal birth. Finally, a small molecule inhibitor of TGF-beta receptors demonstrates its regulation of FGF activity. We propose that FGF signaling serves as a key regulator of neurogenesis in the habenular nuclei, integrating TGF-beta signaling with control of cell cycle in neuronal precursors.

Moreover, great care should be taken to evaluate brain development during pre-clinical testing of compounds affecting these pathways.

Session Role Order: 6
Session Role: Presenter
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Funding: No SOT Funding Needed
Presentation Title: The Role of Histone Deacetylase Inhibition in Mediating the Effects of Developmental Toxicants on Limb Development
Presentation Description: Histone deacetylases (HDACs) play a major role in chromatin remodeling, the regulation of gene expression and cell signaling. Several inhibitors of these enzymes have been shown to increase the incidence of birth defects. However, the mechanisms leading to their teratogenicity and the role of each class of HDACs during normal development are not clear. Valproic acid, a class I and II HDAC inhibitor and an anticonvulsant drug, induces neural tube defects and skeletal malformations *in vivo*. In the *in vitro* murine limb bud culture system valproic acid causes a significant concentration-dependent increase in limb abnormalities and dysregulates the expression of Sox9 and Runx2, two transcription factors that are key in chondrogenesis and osteogenesis in the limb. Valpromide, a close analog of valproic acid and a weak *in vivo* teratogen, does not inhibit HDAC activity and has little effect on either limb morphology or the expression of marker genes. Thus, the effects of valproic acid are correlated with its HDAC inhibitory activity. Subsequent studies on the class-specificity of the effects of HDAC inhibitors in the limb bud culture system have revealed that inhibition of class II HDACs has a minimal impact on limb differentiation; in contrast, inhibition of class I or III HDACs is associated with severe developmental toxicity. Together, these studies indicate that dysregulation of the acetylation of specific target proteins may be important in mediating the action of some developmental toxicants. Supported by CIHR.

Session Role Order: 7
Session Role: Presenter
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Funding: Registration Only
Presentation Title: Elucidation of the Mechanism of Action of Thalidomide and IMiD Compounds Gives Novel Insights Into Their Pleiotropic Effects
Presentation Description: Thalidomide and its analogues, the IMiD compounds Lenalidomide and Pomalidomide have been transformational for the pharmaceutical industry; the thalidomide tragedy altered our perceptions of safety monitoring and transformed the drug regulatory environment. Subsequently the IMiD compounds have now established roles in multiple myeloma, mantle cell lymphoma, and myelodysplastic syndrome. The precise molecular mechanism of action of these drugs, however, has been unknown. A potential unifying key to unraveling the actions of the IMiDs began with

the relatively recent observation that thalidomide caused phocomelia birth defects by binding to cereblon (CRBN), part of a complex with E-3 ubiquitin ligase activity. We solved the structures of CRBN in the apo state and with thalidomide or Pomalidomide and this has given us insights as to why the rodent model did not give the limb malformations seen in other species. In order to identify the amino acids responsible for this difference, we tested a series of human/mouse CRBN chimeric proteins for their ability to confer Lenalidomide-induced activity. Substitution of amino acids in human CRBN with the corresponding amino acid in mouse CRBN revealed only one substitution, V387I (human CRBN isoform 2), that disrupted the Lenalidomide-responsiveness of human CRBN. Remarkably, substitution of the isoleucine at this position in mouse CRBN for the human valine was sufficient to confer Lenalidomide-induced activity in mouse cells. Taken together our data provide important mechanistic insights into the activity of IMiD drugs by enabling the development of novel agents that can modulate CRBN activity in different cellular contexts to gain a broad range of therapeutic activities.