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PRIMARY PRESENTATION CHOICE: Symposium
Secondary Presentation Choice: Workshop

TITLE: Revising Biology: Using Genomic and Epigenomic Editing to Gain Novel Insight into the Molecular Mechanisms of Toxic Exposure Effects and Susceptibility

Primary Endorser: **Molecular and Systems Biology Specialty Section**
Endorser 2: Mechanisms Specialty Section
Endorser 3: Drug Discovery Toxicology Specialty Section

OVERALL ABSTRACT: The genome and epigenome work hand-in-hand as central regulators of cell fate and function and thus serve as key mediators of susceptibility and toxic exposure effects. The use of traditional molecular methods has established a foundation with respect to the molecular mechanisms underlying the adverse effects of many toxic exposures; however, their efficacy in defining causative relationships between gene products, genetic polymorphisms, and epigenetic modification states with toxic exposure effects and susceptibility has been limited. The recent development of practical applications for clustered, regularly interspaced, short palindromic repeat (CRISPR), and Piwi-interacting RNA (piRNA) technology holds the potential to overcome these obstructions by permitting the selective revision of both the genome and epigenome in both toxicology research and clinical applications. CRISPR-mediated gene editing allows for the selective introduction or correction of mutations, deletion of target DNA, or introduction of fluorescent markers/biosensors or epitope tags to endogenous target genes or other loci. Further, piRNAs and deactivated CRISPR-associated protein 9 (dCas9) fusions with enzymes that add or remove epigenetic modifications can be targeted to alter both DNA methylation and histone modifications at specific loci to directly link changes in epigenetic modification states to exposure outcomes and susceptibility. The application of these technologies will open the door to the next generation of precision therapeutics and revolutionize the field of toxicology by providing novel opportunities to understand and modulate exposure-related disease and susceptibility at the genetic and epigenetic level. The goal of this session is to examine the range of applications of genome and epigenome engineering from their use in molecular and mechanistic toxicology studies to their potential as therapeutic strategies; and to review the inherent safety considerations that their use entails. To achieve this we will bring experts together to discuss the development of these technologies and their current use in toxicity studies covering cultured human cells, mouse models, and human clinical trials. We will answer questions such as: How do CRISPR-Cas9-mediated genomic/epigenomic engineering and piRNAs work, and what are the benefits and challenges facing their integration into the field of toxicology? Can CRISPR-Cas9 genomic editing be used to explore the role of key toxicity-associated pathways, such as NF-kB and NRF2, in the response to oxidative stress? How can the targeted modification of epigenetic states with dCas9 and piRNAs be used to provide causative relationships between specific epigenetic loci and disease/exposure outcomes? What is the current state of CRISPR-based therapies and how does the toxicity and efficacy testing of these next-generation pharmaceuticals differ from that used for traditional therapeutic agents? Following the session attendees will have a better understanding for the benefits, challenges, and applications of genome and epigenome engineering approaches in toxicology studies. Further, they will gain perspective on the unique

considerations required during the development and testing of these technologies as next-generation therapeutic agents.

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Presenter 1

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Funding: No SOT Funding Needed
Presentation Title: CRISPR and piRNAs: Fundamental Mechanisms and Key Applications of The Next Generation of Molecular Technologies in the Field of Toxicology
Presentation Abstract: Exploration into the roles of genes, the proteins that they encode, and the functions that they carry out within the cell is a founding pillar in the field of toxicology. Recent breakthroughs in clustered, regularly interspaced, short palindromic repeat (CRISPR) technology and PIWI-interacting RNAs (piRNAs) have resulted in the development of novel approaches to selectively modify both the genome and the epigenome. The application of these technologies will fundamentally revolutionize the development of highly sophisticated genetic and epigenetic models using human cells to examine causal relationships between specific genetic polymorphisms and epigenetic modifications at discrete loci with toxic exposure effects and susceptibility. CRISPR technology directs the nuclease CRISPR-associated protein 9 (Cas9) to the target DNA locus using a sequence-specific guide RNA (gRNA), which provides increased specificity and ease of design compared to previous approaches using zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs). CRISPR can also be used to specifically modify the epigenome through either the targeted disruption of CpG sites that are subject to DNA methylation, or through the expression of epigenetic modifying enzymes/domains that are fused to “deactivated” Cas9 (dCas9), such that it can be targeted to specific genomic loci by synthetic guide RNA (sgRNA) but lacks nuclease activity. This permitted the targeting of transcription factors or epigenetic modifying enzymes to specific genomic loci, thus providing the technical framework to interrogate the relationships between changes in specific epigenetic modifications and exposure-associated phenotypes. Separately, a novel class of small RNAs, known as piRNAs, has recently been shown to orchestrate the targeted methylation of DNA within the mammalian genome. In

a manner similar to the capacity of sgRNA to target dCas9, synthetic designer piRNAs can recruit DNA methyltransferases to hypermethylate specific genomic loci, thus silencing associated genes. This presentation will set the stage for the session by describing the similarities and differences between the molecular mechanisms utilized by these transformative tools, discussing their potential within the field of toxicology, and demonstrating their applications in revising biology.

Presenter 2
Name: Cheryl Rockwell
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Funding: No SOT Funding Needed
Presentation Title: Use of CRISPR/Cas9 to Elucidate the Role of Nrf2 in the Response of T Cells to Electrophilic and Oxidative Stress
Presentation Abstract: The introduction of CRISPR/Cas9 technology for gene editing has transformed basic science research. In our lab we have used CRISPR/Cas9 gene editing to elucidate the role of nuclear factor erythroid 2-related factor 2 (Nrf2) in T cell function. Nrf2 is a transcription factor that is activated by cell stress. It is well-established that the Nrf2 pathway is important for detoxification and metabolism of reactive xenobiotics. Although a number of well-characterized Nrf2 activators have significant effects on the function of human CD4 T cells, the role of Nrf2 in these effects remains unclear. We used CRISPR/Cas9 to knock out expression of Nrf2 as well as the expression of certain proteins known to repress Nrf2 activity, such as KELCH-like ECH-associated protein 1 (Keap1). Using this approach, we have found that Nrf2 activators, such as tBHQ, H2O2 and CDDO-Im have both Nrf2-dependent and –independent effects in T cells. Specifically, we found that inhibition of IL-2 by CDDO-Im, but not tBHQ, was dependent on Nrf2. In addition, CDDO-Im and tBHQ both inhibit expression of the IL-2 receptor, CD25, in a partially Nrf2-dependent manner. We also found that mitochondrial stress and cell death by H2O2 was significantly increased in the Nrf2-deficient cells. Furthermore, our studies demonstrate that in the absence of xenobiotics, Nrf2 negatively regulates IL-2 induction by activated human Jurkat T cells, suggesting an endogenous role for Nrf2 in IL-2 regulation. This novel methodology represents a relatively easy and inexpensive option for reliably modulating gene expression and dissecting the role of transcription factors such as Nrf2, or other genes involved in the response to toxic stimuli. Overall, our studies demonstrate an important role for Nrf2 in modulating T cell function, suggesting activation of Nrf2 could be a useful marker for predicting immunomodulatory potential of chemicals. CRISPR/Cas9 was an important tool for these studies that also has broad applicability for a variety of different mechanistic studies in toxicology.

Presenter 3
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SOT Member: No
Funding: SOT Full Funding

Presentation Title: Applications of CRISPR/Cas9-Based Epigenetic Editing Technologies in Modeling and Treating Human Disease

Presentation Abstract: The ability to define the relationships between endogenous genetic and epigenetic variation and human disease is lacking. This limitation is especially pressing for diseases that are linked to dynamic environmental and/or inflammatory signals, and therefore have multiple potential pathological drivers. The repurposing of prokaryotic CRISPR/Cas9 adaptive immune systems has revolutionized the capacity to engineer the human genome and created tools to meet these critical needs. The nuclease activity of Cas9 can be abolished to produce a "deactivated" genomic targeting platform called dCas9. The dCas9 scaffold can be combined with transcriptional or epigenetic effector domains to finely tune the expression of endogenous genes and artificially deposit epigenetic signatures. We have developed a novel epigenome editing tool by fusing the catalytic core of the human EP300 acetyltransferase to dCas9 (dCas9-p300). We have used this programmable acetyltransferase to demonstrate the causal linkage between endogenous chromatin acetylation and subsequent gene expression. Moreover, we have shown that dCas9-p300 robustly activates gene expression from endogenous enhancers and promoters, thus expanding the targeting capacity of epigenome editing tools. Additionally, we have developed a high-throughput epigenome editing platform to functionally interrogate the noncoding human genome. Importantly, these epigenome editing strategies can be used to assign the function of epigenetic signatures and to activate or suppress the transcription of genes putatively involved in disease phenotype, and have the potential to modulate exposure effects and susceptibility at the epigenetic level. For instance, our group has used these approaches to activate and suppress loci involved in human disease and inflammation, including the human globin genes, TNFR1, and IL1R1. Cutting-edge CRISPR/Cas9-based genome and epigenome engineering technologies will continue to impact basic and translational research into the foreseeable future. This talk will provide an overview of the principles that govern the use of these tools in establishing molecular mechanisms underlying human disease and in exploring cellular responses to toxic exposures and inflammation. In addition, the potential therapeutic applications of these technologies in clinical settings will be discussed.

Presenter 4

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Funding: No SOT Funding Needed
Presentation Title: Development of piRNAs for Target-Specific DNA Methylation

Presentation Abstract: Epigenetic changes to DNA are associated with age, disease, and environmental influences. Precision modification of the epigenome holds great promise for our ability to modify environmentally induced changes in gene expression, yet is currently out of reach using common techniques (drugs, transgenics, etc.). Through a NIH Director's Transformative Award we are developing a suite of tools, based on the Piwi-interacting RNA (piRNA) system, to advance precision epigenetic editing, while avoiding drawbacks of current technology (e.g. off-target effects). Until recently, it was widely believed that Piwi Like RNA-Mediated Gene Silencing (PIWIL) gene expression was confined to the germ line of animals, and neither PIWILs nor piRNAs were present or active in somatic tissues. Our

research overturns this accepted knowledge by finding widespread PIWIL expression in multiple somatic tissues of the mouse in three male biological replicates in whole brain, hippocampus, heart, kidney, liver and testes, and one female biological replicate in ovary. While no tissue exhibited expression of any PIWIL as high as testes, the considerable somatic PIWIL gene expression within the mouse confirms piRNA function in the soma of adults. Thus, we are now using this class of RNA to develop in-vivo technology to target specific genes and loci for stable, mitotically heritable, silencing at pre-determined genomic locations. Using the agouti viable yellow mouse, which varies in coat color concomitantly with DNA methylation at a single locus, we use piRNA to induce DNA methylation of the Avy transposon, providing direct visual semi-quantitative evidence of systemic molecular silencing at this locus. This research is providing sorely needed evidence clarifying the roles and activity of piRNA in somatic tissues of mammals and will be used to develop piRNA targeted methylation for the wider toxicological research and therapeutic communities.

Presenter 5

Name: Monika Chabicovsky
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SOT Member: No
Funding: SOT Full Funding
Presentation Title: Leading the Edge: Toxicity and Safety Testing with CRISPR/CAS-Based Therapeutics

Presentation Abstract: Clustered regularly interspaced short palindromic repeat (CRISPR)-associated nuclease Cas9 is uniquely positioned to cure diseased cells and tissues via genome editing; leading either to the removal or the correction of mutations by providing an exogenous wild-type DNA template. As of now, most genome editing therapeutic approaches have focused on the potential treatment of serious monogenic diseases; however, the therapeutic application is beginning to broaden to less severe indications and larger patient populations and therefore, an even more diligent benefit-risk assessment is required for a responsible and rationale pre-clinical development of these products. This presentation will focus on critical considerations for non-clinical development of gene-editing product candidates and will compare the key elements and endpoints of a pivotal non-clinical safety program for a gene edited product with a standard program of small and large molecules. Potential risks related to CRISPR/CAS-edited products, among others the potential for cyto- and genotoxicity driven by “off-target” effects such as double-stranded breaks at unintended genetic loci, gene-rearrangements, translocations or immunogenicity, and immunotoxicity caused by the bacterial Cas9 protein will be described and discussed in the context of US and EU authority expectations. In addition, typical gene-medicine related challenges like the definition of a safe human starting dose or the selection of relevant animal models to realize their clinical translation will be addressed as well.

Presentation 6: Panel Discussion/Q&A