CONTROL ID: 12345
PRESENTATION TYPE: CE Basic
Secondary Pres Type - Proposal:
TITLE: PROCESS BASED APPROACHES TO MODULATING GENE AND PROTEIN EXPRESSION IN VIVO AND IN VITRO
CATEGORY: Molecular Biology, Mechanisms
CONTACT (NAME ONLY): James Gordon
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ABSTRACT BODY: Abstract Body (Proposal Submission): The mechanistic analysis of cellular responses to xenobiotics requires the ability to modulate important genes involved in specific pathways. Such genes include those that encode receptors that associate with xenobiotics as well as the enzymes involved in xenobiotic metabolism. The ability to modulate these genes and proteins in vitro and in vivo has become accessible to more laboratories with the refinement of techniques such RNA interference (RNAi), viral gene delivery, morpholino-mediated gene knock down and targeted gene disruption. However, the ability to utilize these techniques and generate reproducible results requires a detailed understanding of the advantages and applications of each procedure. Thus, the goal of this course is to provide the investigator with an overview of experimental design and the use of proper controls for four cutting edge techniques. The first talk will focus on experimental design and analysis of RNAi to reduce endogenous target proteins in culture cells with emphasis on controls and endpoint analysis. The second presentation will move to the zebrafish model system and discuss the use of morpholino-mediated gene knock down to reduce the expression of specific proteins in embryos. The third presentation will discuss gene delivery utilizing the adenovirus system for reduction of gene expression in mice. The forth presentation will detail the use or transgenic approaches in mouse models to modulate the expression of specific target genes or knock-in genes from other species. This course should be of broad interest to laboratories considering a mechanistic approach to understanding signal transduction pathways, gene expression and protein-protein interactions as well as those currently investigating these endpoints.

CHAIR/PRESENTER INFORMATION:
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Role: Chair

Member Type: SOT Member
Funding: No SOT funding required
Presentation Title: Use of siRNA Technology to Modulate Gene Expression in Culture Cells
Presentation Description: The modulation of endogenous protein expression in cell culture models is a powerful system for rapidly assessing the function of a target protein prior to embarking on extensive experiments in whole animals. The use of RNA interference (RNAi) by using various types of interfering RNA molecules has revolutionized the ability to quickly reduce protein expression in any cell line. However, the ability to get consistent reductions in target protein expression that impact downstream gene regulatory events requires optimization, and the ability to assess physiologically relevant endpoints. This presentation will cover the advantages and disadvantages of the different types of RNAi techniques that are now available, transfection options for both transient and stable expression of RNAi molecules, and techniques for the analysis of target protein expression in cells. Special attention will be given to the use of proper controls and how investigators can be sure that results represent response of the entire cell population.

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Role: Presenter 2
Member Type: SOT Member
Funding: No SOT funding required
Presentation Title: Use of Morpholinos to Modulate Gene Expression in Zebrafish
Presentation Description: Global gene expression analysis is a powerful approach to evaluate molecular responses to toxicant exposures, but the true goal is to identify the gene expression changes that are causally related to observed toxic responses. The key question is what are the proximal gene expression changes that precede overt signs of toxicity? All model platforms are amenable to microarray analysis; a unique advantage of embryonic zebrafish is that it is feasible and routine to rapidly evaluate the role of individual genes in vivo using antisense morpholinos (MO) gene repression. MO can be used to effectively repress gene expression during the early stages of zebrafish development and this approach is well suited for molecular toxicological evaluations. The presentation will illustrate the principle of MO gene repression, will discuss MO design, MO targeting, and MO delivery. The important consideration of the

strengths and limitations of the technology and reagents will also be discussed.

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Role: Presenter 3
Member Type: SOT Member
Funding: No SOT funding required
Presentation Title: Adenovirus-Mediated Gene Delivery to Modulate Protein Expression In Vivo
Presentation Description: The ability to transfer DNA rapidly and efficiently into transformed cell culture models in vitro has greatly facilitated molecular studies into gene and protein function. However, classical transfection techniques are generally not amenable to gene transfer into primary cell types. Recombinant adenoviruses provide an efficient means of gene transfer into a wide variety of primary cells in vitro and in vivo. Significantly, infusion of adenovirus directly into the peripheral circulation preferentially target hepatocytes in vivo, and provides a facile mechanism to alter liver protein expression. This presentation will detail the generation of recombinant adenoviruses encoding proteins and small hairpin RNAs (shRNA) and their use to alter hepatic protein expression in mice. The presentation will highlight the strengths and limitations of the technology and reagents involved using actual data obtain in our studies examining Ah Receptor function. The adenovirus system complements existing strategies using transgenic and knockout mice and may even provide certain advantages over the latter.

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Role: Presenter 4

Member Type: Non-member

Funding: SOT Full Funding Requested

Presentation Title: Transgenic Approaches to Modulate Gene Expression in Mouse Models

Presentation Description: Genetic modification of mice is a valuable tool to determine the mechanisms of chemical toxicity and a means to create models organisms for human risk assessment. In particular, targeted disruption of enzymes that metabolic activate toxicants and carcinogens, and receptors that mediate the toxic responses has proven of great value in toxicology research. Most genes encoding P450s involved in xenobiotic metabolism can be disrupted in the embryo and the resulting mice display no deleterious phenotypes making them ideally suited to study the biological effects of chemicals. Xenobiotic receptors such as the pregnane X receptor (PXR), constitutive androstane receptor (CAR), and peroxisome proliferators-activated receptor α (PPARα) can also be disrupted without compromising development or physiological homeostasis. In other cases where loss of a gene produced a phenotype, conditional null mice should be generated. Transgenic “humanized” mice can also be produced in which the mouse gene is replaced by the corresponding human gene in an effort to produce an animal model that would more accurately predict human metabolism and toxicity. The practical aspects of development and use of mice of mice in toxicology research will be discussed.