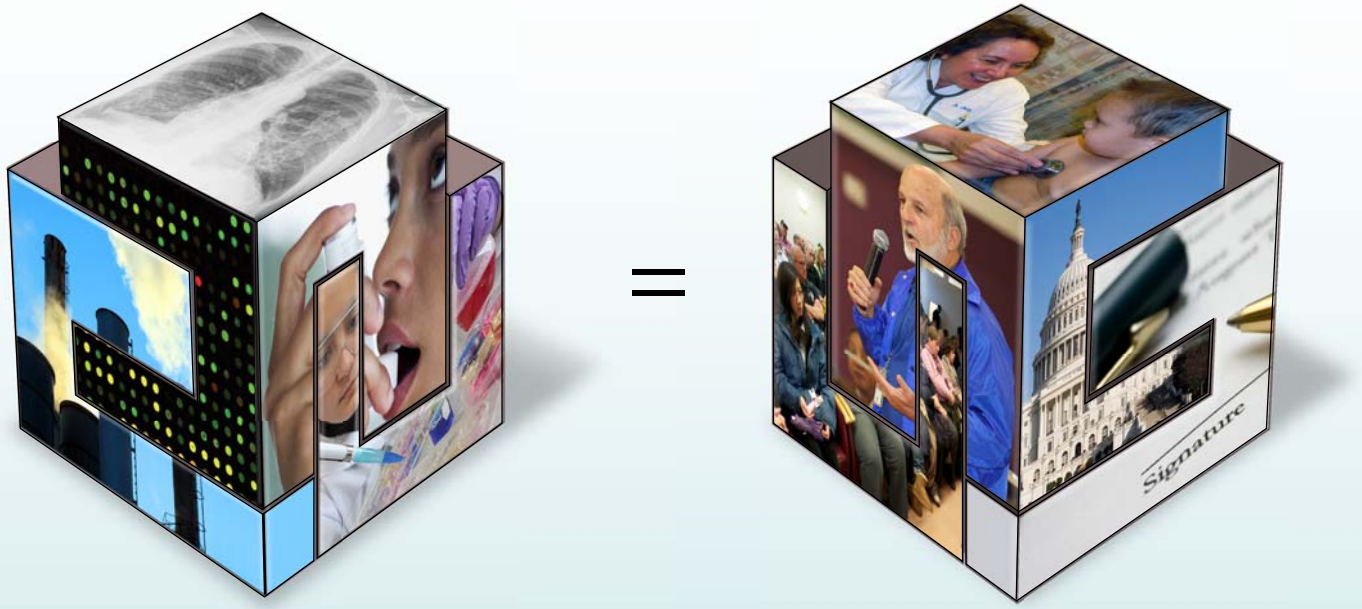
Center of Excellence in Environmental Toxicology

Seventh Annual CEET Symposium

Environmental Health in Translation

Friday, May 17, 2013
Villanova Conference Center



Center of Excellence in Environmental Toxicology (CEET)

SEVENTH ANNUAL CEET SYMPOSIUM

Environmental Health in Translation

Villanova Conference Center
May 17, 2013

Host Institution
CENTER OF EXCELLENCE IN ENVIRONMENTAL TOXICOLOGY
PERELMAN SCHOOL OF MEDICINE AT THE UNIVERSITY OF PENNSYLVANIA

Perelman School of Medicine University of Pennsylvania | CEET
CENTER OF EXCELLENCE IN ENVIRONMENTAL TOXICOLOGY

NIH National Institute of Environmental Health Sciences

Contents

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BREAKFAST AND REGISTRATION

SESSION 1 THE AMBLER COMMUNITY AND ASBESTOS EXPOSURE

8:30 – 9:00 A.M. **Superfund Research Program: Asbestos Fate, Exposure, Remediation and Adverse Health Effects**
Ian A. Blair, PhD
A.N. Richards Professor of Pharmacology
Perelman School of Medicine University of Pennsylvania

9:00 – 9:30 A.M. **Preclinical Animal Models of Mesothelioma**
Joseph Testa, PhD
Carol & Kenneth E. Weg Chair in Human Genetics
Fox-Chase Cancer Center

9:30 – 10:30 A.M. BREAK AND POSTERS

SESSION 2 HEAVY METAL EXPOSURES

10:30 – 11:00 A.M. **Lead Exposure and Children's Neurobehavioral Outcomes**
Jianghong Liu, PhD, RN, FAAN
Associate Professor of Nursing

11:00 – 12:00 A.M. **Environment, Epigenetics and Fetal Growth**
Keynote Lecture 1: ***Robert O. Wright, MD, MPH***
Professor of Preventive Medicine and Pediatrics, Director of the Division of Environmental Health, Deputy Director of the Children's Environmental Health Center, Icahn School of Medicine at Mount Sinai

12:00 – 1:30 P.M. LUNCH

SESSION 3 ENABLING TRANSLATION IN ENVIRONMENTAL HEALTH

1:30 – 2:00 P.M. **Integrative Health Sciences Facility Core and Human Exposures: Successes and Challenges**
Rey Panettieri, Jr. MD
Robert L. Mayock and David A. Cooper Professor of Medicine
Director, Airways Biology Initiative

Howard Kipen, MD, MPH
Director of Occupational Health, Professor of Environmental and Occupational Medicine, Robert Wood Johnson Medical School

2:00 – 2:30 P.M. **Translational Biomarker Core In Action**
Clementina Mesaros, PhD
Technical Director

2:30 – 3:00 P.M. **Opportunities for Genetics and Informatics to Improve Environmental Health**
Ben Voight, PhD
Assistant Professor of Pharmacology

3:00- 3:30 P.M. **Community Outreach and Engagement-New Initiatives**
Marilyn Howard
Adjunct Associate Professor of Occupational and Environmental Medicine
CEO, Howarth Consulting

3:30 – 4:30 P.M. BREAK AND POSTERS

4:30 – 5:30 P.M. **Impact of Genome on Metabolome**
Keynote Lecture 2: ***Professor Jurek Adamski***
Head of Department, Genome Analysis Centre, Institute of Experimental Genetics
Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH)

5:30 – 6:30 P.M. WINE AND CHEESE RECEPTION

Keynote Speakers

Dr. Robert Wright is a pediatrician, epigeneticist, and environmental epidemiologist at Mount Sinai School of Medicine, Department of Preventive Medicine. He is the Department Vice Chairman and Director of the Division of Environmental Health, and former director of the Harvard Superfund Research Program. He is the Principal Investigator of an ongoing birth cohort in Mexico City (Early Life Exposures in Mexico and ENvironmental Toxicology-ELEMENT) in collaboration with the National Institute of Public Health, Mexico and the National Institute of Perinatology. This cohort is studying interactions between metals and psychosocial stress (such as exposure to violence). He also founded the (Metals Assessment Targeting Community Health) MATCH study in Tar Creek, Oklahoma. His research expertise is in the field of gene-environment interaction in child development. He has published over 120 papers, most of which deal with Environmental Health and has served on numerous national committee/advisory boards in the field of Pediatric Environmental Health. Dr. Wright directs the Molecular Environmental Health Lab at Mount Sinai. He is the Research Director of the Region 2 Pediatric Environmental Health Subspecialty Unit and member of the American Academy of Pediatrics Committee on Environmental Health.

Jerzy Adamski is Head of Genome Analysis Center at Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH) which integrates platforms of genomic, transcriptomic, proteomic and metabolomic research, promoting high throughput research in mechanisms of the development and progression of complex diseases. Professor Adamksi obtained his PhD in Endocrinology from Medical Academy and Polish Academy of Sciences, Poznan, Poland and completed his Postdoctoral training and subsequent Habilitation University of Hannover, Max-Planck-Institute for Experimental Endocrinology. His interest is to identify the factors responsible for the pathogenesis of complex metabolic diseases such as diabetes, obesity, cardiovascular disorders and diseases of environmental etiology such as asthma. He discovered the molecular basis of a sub-type of Zellweger syndrome and analyzed mechanisms of steroid hormone pre-receptor control on DNA, protein and metabolite levels. His laboratory discovered several genes pivotal in steroid and lipid metabolism including human 17beta-HSD types 4, 7 and 14. Recently, his group published the first GWAS study that was correlated with metabolomics (mGWAS). Using this approach his group discovered the impact of SNPs on genetically determined metabotypes in normal and disease-overt humans. He published over 200 papers in peer-reviewed journals and acts as Editor-in-Chief for Journal of Steroid Biochemistry and Molecular Biology.

MISSION AND VISION STATEMENT

The Center of Excellence in Environmental Toxicology (CEET) was launched in 2005 and receives grant support from the National Institute of Environmental Health Sciences. It is one of only twenty designated Environmental Health Sciences Core Centers in the nation.

The CEET elucidates the mechanistic links between environmental exposures and human disease and translates its findings into action to improve the health of vulnerable individuals, and local, national and global communities.

The CEET marries its relevant research excellence to tackle the environmental challenges that may represent an assault on our public health. Many of these challenges have their origins in community-based concerns. Examples include the hazard presented by petrogenic polycyclic aromatic hydrocarbons from the Deepwater Horizon oil-spill in the Gulf of Mexico; the fate, transport, remediation and adverse health effects of asbestos exposure in the Ambler Community in SE. Pennsylvania (which is home to one of the largest Superfund Asbestos hazardous waste sites in the country); and natural gas drilling operations in the Marcellus Shale, where citizens are concerned about the effects of air-pollution and water contamination on their health.

The CEET has research excellence in these and related themes. Its Affinity Group in Lung and Airway Disease examines the relationship between poor air quality and air pollution in our region (ozone, fine particulate matter, allergens, SO_2, NO_2 and CO emissions) and disease (asthma, luing cancer, mesothelioma and COPD); Its Affinity Group in Reproduction, Endocrinology, and Development examines the relationship between exposures in windows of susceptibility and health outcomes. Investigators explore the association between in uetro exposures, epigenetic imprinting and the developmental basis of adult disease. These organ-based themes are linked to our Affinity Groups in disease mechanism, which include Oxidative Stress and Oxidative Stress Injury and Gene-Environment Interactions.

The CEET enables its investigators to conduct exposure science using its Translational Biomarker Core which uses sophisticated liquid chromatography mass spectrometry methods to identify and develop assays of biomarkers of exposure and effect, and measure changes in targeted and unbiased metabolomes following in response to exposures, disease onset and progression. The CEET also maintains a bioinformatics core so that large siloed data bases in genomics, proteomics and metabolomics can be merged as reporter of system wide responses and adaptation to environmental exposure.The Integrated Health Sciences Facility Core (IHSFC) of the CEET provides assistance with a broad range of transdisciplinary services including study design, enrollment of research subjects, human, population and community exposures, , access to biospecimens via a CEET biorepository and data management, and genetic and non-genetic biostatistical analyses.

The CEET engages select communities to empower them with new knowledge so that they are better informed to tackle issues of environmental health threats, health disparities and environmental justice. To improve the environmental health of these and similar affected communities, the CEET is actively involved in the education of health care professionals (Residency Program in Occupational and Environmental Health, Nursing concentration in Occupational and Environmental Health, and Masters of Public Health Programs).

The CEET also disseminates findings to all stakeholders including community organizations, local, state and federal officials and agencies (Pennsylvania Department of Health, Pennsylvania Department of Environmental Protection, Environmental Protection Agency) to affect change in environmental health and public health policies.

The CEET is a flexible entity that marshals excellence in basic, translational, patient-oriented and population-based research in the School of Medicine and Children's Hospital of Philadelphia. Although primarily housed in the School of Medicine, the 58 CEET Investigators belong to 16 departments and five schools at the University of Pennsylvania.

CENTER OF EXCELLENCE IN ENVIRONMENTAL TOXICOLOGY

University of Pennsylvania School of Medicine

ADMINISTRATIVE CORE

Director: Trevor Penning, Ph.D.

Deputy Director: Reynold Panettieri, M.D.

Affinity Group I
OXIDATIVE STRESS AND
OXIDATIVE STRESS INJURY

Director: Ian Blair, PhD
Paul Axelsen, MD
Joseph Baur, PhD
Michael Beers, MD
Jeffrey Field, PhD
Aron Fisher, MD
Garret FitzGerald, MD
Toshinori Hoshi, PhD
Harry Ischiropoulos, PhD
Kelly Jordan-Sciutto, PhD
Vladimir Muzykantov, MD/PhD
Trevor Penning, PhD
Richard Schultz, PhD
Rebecca Simmons, MD
Andrew Strasser, PhD
Stephen Thom, MD, PhD

Affinity Group II
REPRODUCTION, ENDOCRINOLOGY,
AND DEVELOPMENT (READ)

Director: George Gerton, PhD
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Marisa Bartolomei, PhD
Shelley Berger, PhD
Samantha Butts, MD, MSCE
Ted Emmett, MD, MS
Struan Grant, PhD
Brett Kaufman, PhD
Karen Knudsen, PhD
Michael Levine, MD
Jianghong Liu, PhD/RN
Ralph Meyer, PhD
Sarah Millar, PhD
Mary Mullins, PhD
Katherine Nathanson, MD
Sam Parry, MD
Trevor Penning, PhD
Sara Pinney, MD, MS
Richard Schultz, PhD
Rebecca Simmons, MD
Virginia Stallings, MD
Sindhu Srinivas, MD/MSCE
Jeremy Wang, MD/PhD

Affinity Group III
LUNG AND AIRWAY DISEASE

Director: Michael Beers, M.D
Steve Albelda, MD
Andrea Apter, MD, MSc
Jason Christie, MD, MSCE
Melpo Christofidou-Solomidou, PhD
Pamela Dalton, PhD
Richard Doty, PhD
Angela Haczku, MD/PhD
Vera Krymskaya, PhD
Rey Panettieri, MD
Trevor Penning, PhD
Anil Vachani, MD

Affinity Group IV
GENE-ENVIRONMENT INTERACTIONS

Director: Alexander S. Whitehead, DPhil
Marisa Bartolomei, PhD
Shelley Berger, PhD
Ian Blair, PhD
Jinbo Chen, PhD
Youhai Chen, MD/PhD
Jason Christie, MD, MSCE
Struan Grant, PhD
Hakon Hakonarson, MD/PhD
John Hogenesch, PhD
Todd Lamitina, PhD
Hongzhe Li, PhD
Sarah Millar, PhD
Katherine Nathanson, MD
Jennifer Pinto-Martin, PhD/MPH
Trevor Penning, PhD
Timothy Rebbeck, PhD
Virginia Stallings, MD
Sarah Tishkoff, PhD
Aalim Weljie, PhD

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MOLECULAR PROFILING CORE

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Toxicogenomics
Associate Director: John Tobias, PhD

Toxicoproteomics
Associate Director: Chao-Xing Yuan, PhD

Biomarker
Associate Director: Clementina Mesaros, PhD

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Human Studies Design and Performance Services
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Population Exposure Services
Associate Director: Ted Emmett, MD, MS

CEET Biorepositories
Associate Director: Rey Panettieri, Jr., M.

Biostatistics
Associate Director: Kathleen Propert, ScD
Genetics Statistician: Mingyao Li, PhD

COMMUNITY OUTREACH AND ENGAGEMENT CORE

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Deputy Director: Richard Pepino, MS

Andrea Apter, MD, MSc
Charles Branas, PhD
Pamela Dalton, PhD
Ted Emmett, MD, MS
Jeffrey Field, PhD
Ira Harkavy, PhD
Jianghong Liu, PhD, RN
Judith McKenzie, MD, MPH
Kevin Osterhoudt, MD, MSCE
Trevor Penning, PhD
Jennifer Pinto-Martin, PhD, MPH
Alexander S. Whitehead, PhD

Oxidative Stress and Oxidative Stress Injury

O1 Metabolism and Protein Adduction of a Cyclooxygenase-2/15-prostaglandin Dehydrogenase Derived Product from Arachidonic Acid.

Nathaniel Snyder, Alejandro A. Pacheco, Suhong Zhang, Ian Blair
Centers for Cancer Pharmacology and Excellence in Environmental Toxicology, University of Pennsylvania Perelman School of Medicine

Counter regulation of arachidonic acid (AA) metabolizing enzymes cyclooxygenase-2 (COX-2) and 15-prostaglandin dehydrogenase (15-PGDH) has been observed in multiple cancers and implicated in inflammatory pathologies. Interestingly, COXs also mediate the metabolism of AA to 11-hydroxyeicosatetraenoic acid (HETE), which can then be oxidized by dehydrogenases to the corresponding keto-metabolite, 11-oxo-eicosatetraenoic acid (oxo-ETE). 11-oxo-ETE has been identified from human isolates yet currently has no known receptors or known physiologic function. However, polyunsaturated molecules possessing an alpha,beta-unsaturated ketone moiety may undergo saturation, as well as form adducts to glutathione, cysteine, and histidine. Therefore, this study was designed to explore the metabolism and protein adduction of 11-oxo-ETE.

The methyl ester of 11-oxo-ETE was found to be enriched in the intracellular fraction of HUVECs, and thus was used as targeted delivery to study the metabolism and biologic effect of 11-oxo-ETE. Using an untargeted metabolomics approach to identify differentially abundant features in a treated versus control group of HUVECs, multiple metabolic pathways were identified. Saturation of double bonds and GSH-adducts were confirmed by LC-MS^2 experiments. Using the tryptic peptide of the DNA binding domain of the p50 subunit of the transcription factor NF-kappaB, a cysteine and a histidine lipid-protein adduct was identified by LC-MS/MS. MS^n experiments were used to support the identity of the adduct. Electrophoretic mobility shift assays and a luciferase reporter assay were used to show inhibition of induced p50/p65 signaling by treatment with 11-oxo-ETE.

Supported by NIH grant P30ES013508

O2 Analysis of all Citric Acid Cycle Metabolites by Liquid Chromatography – Tandem Mass Spectrometry

Andrew Worth, Sankha Basu, Clementina Mesaros, Nathaniel Snyder, Ian Blair
Centers for Cancer Pharmacology and Excellence in Environmental Toxicology, University of Pennsylvania Perelman School of Medicine

Mitochondrial dysfunction has been linked to neurodegenerative diseases. Despite the use of mitochondrial inhibitors as neurodegenerative models in rodents, there remains a lack of understanding of the metabolic alterations that occur upon pharmacologic disruption of cellular respiration. Implementing isotopically labeled metabolic substrates coupled with isotopomer analysis gives insight into the utilization of nutrients throughout energy producing metabolic pathways. A demanding prerequisite of performing isotopomer analysis is the need for superior sensitivity to allow for the quantification of a metabolite's isotopic distribution. Therefore, described here is a novel chemical derivatization scheme to allow for highly sensitive detection of the organic acids in the citric acid cycle. This affords great insight into compensatory metabolic alterations that occur upon mitochondrial dysfunction, shedding light on early mechanistic causes of neurodegeneration.

Oxidative Stress and Oxidative Stress Injury

O3 Asbestos Fate, Exposure, Remediation, and Adverse Health Effects

Trevor M, Penning, Ian A. Blair
Centers for Cancer Pharmacology and Excellence in Environmental Toxicology, University of Pennsylvania Perelman School of Medicine

Abstract: The University of Pennsylvania Superfund Research and Training Program Center (Penn SRP Center) evolved as a direct consequence of concerns from the community living proximal to the BioRit Asbestos Superfund site in Ambler, PA. The community at Ambler has actively participated in the design of projects to be tackled by the Center. As a result, the Center will consist of two Environmental Science Projects and four Biomedical Science Research Projects, which will be underpinned by one Research Core and four service Cores. Environmental Science Projects 1 and 2 are entitled "Remediation of Asbestos Particles" and "Mobility and Fate of Asbestos Particles," respectively. Biomedical Science Projects 3-6 are entitled "Social Determinants of Risk And Attitudes about Asbestos in a Superfund Environmental Justice Community," "Animal Models of Mesothelioma," "Chemoprevention of Asbestos-Induced Lung Diseases," and "Biomarkers of Asbestos Exposure" The Cores comprise an Administrative Core (Core A), the Community Engagement Core (Core B), the Research Translation Core (Core C), the Biostatistical Research Support Core (Core D), and the Interdisciplinary Training Core (Core E). Advanced techniques for the detection, assessment, and evaluation of the effect on human health of hazardous substances will involve the development of a new animal model of asbestos-induced mesothelioma. Methods to assess the risks to human health presented by asbestos will involve novel metabolomics methodology. A basic biological method to be employed for reducing the amount and toxicity of asbestos will involve the mycorrhizal fungus-mediated conversion of asbestos to a non-toxic molecular form.

Supported by P30ES013508.

O4 Structural Changes in Surfactant Protein D (SP-D) in the Mouse Lung in Response to Ozone (O_3) and Allergen Exposure

Satish Sharma[1], Cynthia Koziol-White[1], Suhong Zhang[2], Chao Yuan[2], Paul Axelsen[3,4], Angela Haczku[1,4]
[1]Pulmonary, Allergy and Critical Care Division, University of Pennsylvania, [2]Department of Pharmacology, Proteomics Core, University of Pennsylvania, [3]Department of Pharmacology,University of Pennsylvania, [4]Center of Excellence in Environmental Toxicology, University of Pennsylvania Perelman School of Medicine

Background: O_3 promotes exacerbation of the asthmatic airway inflammation, likely due to failure of protective immune mechanisms. SP-D is a critical pulmonary innate immune regulator with both suppressive and proinflammatory effects. We hypothesized that exposure to allergen or O_3 induce oxidative modifications in key areas of the SP-D molecule that can induce a functional anti-inflammatory to proinflammatory switch.

Methods: Balb/c mice were sensitized and challenged with Aspergillus fumigatus (Af) and four days later exposed to O_3 (3.0 ppm for 2 h). The bronchoalveolar lavage (BAL) supernatant was assessed for cellular and molecular inflammatory changes 12h later. SP-D was studied by native gel electrophoresis and biotin switch assay using an in-house biotinylated monoclonal anti-SP-D. Recombinant SP-D was treated with air or O_3 (3.0 ppm for 30 min). Bands were extracted from BAL run on SDS-PAGE gels, digested with trypsin and analyzed using Nano LC/MS/MS with Sequest and Scaffold software. In additional experiments recombinant SP-D was denatured, reduced and alkylated before in-solution or in-gel trypsin digestion with or without PNGase F deglycosylation and analyzed using TSQ Vantage and LTQ Orbitrap.

Results: In the BAL of O_3 and Af exposed mice airway inflammation was associated with de-oligomerisation of the SP-D dodecamer. Because presence of nitrosothiols directly reflects cysteine modifications, we used the biotin switch method to investigate nitrosylation of SP-D cysteines and found that SP-D was indeed nitrosylated in response to Af or Af+O_3 inhalation. To verify location of the modified cysteines we used LTQ Mass

Oxidative Stress and Oxidative Stress Injury

spectrometric analysis of trypsinized BAL samples. With this technique however we were able to reveal peptides only in the C-terminal region with coverage of up to 49%. The modifications observed (oxidation and deamidation) were present in controls as well as in O_3 exposed SP-D samples with no differences between the BAL from naïve and treated mice. Additional cyanogen bromide treatment of SP-D after trypsin also failed to reveal N-terminal peptides that were only uncovered after de-glycosylation of the samples. Targeted analysis verified peptide recognition covering both N-terminal cysteines (SVPNTCTLVMCSPTENGLPG R, 726.01 da, Z=3).

Conclusions: Cysteine residues in strategic positions for example at 15 and 20 in the N-terminal region of the SP-D molecule are protected by post translational glycosylation. In spite of this, O_3-induced exacerbation of allergic airway inflammation in mice was associated with cysteine nitrosylation of SP-D in the BAL. This modification maybe responsible for the de-oligomerisation of the native SP-D structure.

Funding: R01AI072197; RC1ES018505; P30ES013508

O5 Metabolism of the Arachidonic Acid Metabolites 11-oxo-ETE and 15-oxo-ETE

Alejandro D. Arroyo, Nathaniel W. Snyder, Suhong Zhang, and Ian A. Blair
Centers for Cancer Pharmacology and Excellence in Environmental Toxicology, University of Pennsylvania Perelman School of Medicine

Introduction: Arachidonic acid (AA) is an important signaling molecule because it serves as the precursor to a wide variety of bioactive lipid products generated through physiologically important metabolic pathways. The cyclooxygenase (COX) pathway is responsible for the metabolism of AA to 11- and 15-hydroxyeicosatetraenoic acid (HETE), which can undergo oxidation of the hydroxyl moiety to a ketone resulting in 11- or 15-oxo-eicosatetraenoic acid (oxo-ETE). The up-regulation of COX-2 and down-regulation of 15-hydroxyprostaglandin dehydrogenase (15-PGDH) is hypothesized to lead to a lipid profile favoring increased angiogenesis, increased proliferation, and chronic inflammation. This counter regulation is a hallmark of multiple human cancers, as well as acute and chronic inflammatory disorders. Both 11-oxo-ETE and 15-oxo-ETE have been identified from human isolates, yet currently have no known receptors or well described metabolism. However, similar polyunsaturated fatty acids undergo extensive biotransformation including; saturation, oxidation, and, conjugation.

Methods: An untargeted liquid chromatography-mass spectrometry (LC-MS) approach was used for identification of novel metabolites as generated by human umbilical vein endothelial cells (HUVECs) and colonic adenocarcinoma (LoVo) cells from the oxo-ETEs. Cells were treated with the parent fatty acids, 11-oxo- and 15-oxo-ETE, as well as the methyl ester derivatives or a vehicle control. LC-MS/MS analysis was used to determine the structure of the molecules. Metabolites identified from the pilot experiment were quantified over a time course experiment.

Preliminary data: With the untargeted approach, several metabolites were detected. Structures of two metabolites were putatively identified by MS/MS as sequential saturations of double bonds. Additional confirmation was obtained from retention time on reversed phase LC. Future experiments will include confirmation of the identity of metabolites by total synthesis of the pure compounds, as well as validation of a method to quantitatively measure absolute amounts of metabolites generated.

Supported by NIH grant P30ES013508

Reproduction, Endocrinology, and Development (READ)

R1 Peripubertal Exposure to High Fructose Corn Syrup Leads to the Later Development of Obesity and Metabolic Abnormalities

Shazia Bhat [1, 2], Rexford Ahima[4], Rebecca Simmons[1,2,3]

[1]Children's Hospital of Philadelphia, Philadelphia, PA, [2]Center for Research on Reproduction and Women's Health, University of Pennsylvania, [3]Center of Excellence in Environmental Toxicology, University of Pennsylvania Perelman School of Medicine, [4]University of Pennsylvania

Background: Sugar consumption in children has increased dramatically in the recent decades, largely in the form of high fructose corn syrup (HFCS). Diets high in fructose have been linked to the development of obesity and hepatic steatosis. It is not known whether exposure to HFCS only during puberty has long-term adverse metabolic effects.

Design/Methods: At weaning, C57/Bl6 mice were given water containing HFCS (50% solution), or no added sugar for 5 weeks. All mice had ad lib access to standard chow. Hepatic metabolomic profiling studies were performed after 3 weeks. GTTs were performed at 10 weeks and body fat content was assessed at 30 weeks.

Results: After 3 weeks of HFCS exposure, body fat content was significantly higher in males and females compared to controls ($p<0.05$). Hepatic metabolomic profiling showed decreases in the intermediates of glycolysis and gluconeogenesis in both sexes ($p<0.05$). In contrast, hepatic monounsaturated fatty acids were increased in both sexes, with a higher increase seen in the HFCS females compared to males ($p<0.05$). Of note, saturated fatty acid levels were significantly elevated in HFCS females but not males. Interestingly, branched chain amino acids were lower in HFCS females compared to HFCS males ($p<0.05$). Hepatic glutamine was higher in HFCS females compared to controls, and glutamate was higher in HFCS males compared to controls ($p<0.05$). The HFCS males were glucose intolerant compared to controls at 10 weeks of age ($p<0.05$) with no change in body fat content compared to controls at 30 weeks of age. The females did not show glucose intolerance, but body fat content remained increased at 30 weeks of age compared to controls ($p<0.05$).

Conclusions: Peripubertal ingestion of HFCS in early life alters body fat content and hepatic metabolism of carbohydrates and fatty acids in male and female mice with sex-specific effects. Males develop insulin resistance without obesity and despite having a high body fat content females do not develop insulin resistance. Low hepatic branched chain amino acids and high glutamine in females exposed to HFCS may be markers for metabolic pathways that protect against the development of insulin resistance.

R2 Development of Stable Isotope Dilution Liquid Chromatography Tandem Mass Spectrometry (LS-MS/MS) Methods for the Determination of the Androgen Metabolome in Serum Following Androgen Deprivation Therapy

Daniel Tamae[1], Steven P. Balk[2], Peter S. Nelson[3], Ian Blair[1]

[1]Center of Excellence in Environmental Toxicology and Cancer Pharmacology, University of Pennsylvania Perelman School of Medicine, [2]Harvard Medical School, Dana-Farber/Harvard Cancer Center and Beth-Israel Deaconess Medical Center, Boston, MA, [3]Division of Clinical Research, Fred Hutchinson Cancer Research Center, Seattle, WA

Androgen deprivation therapy (ADT) is a cornerstone of prostate cancer treatment. However, after initial success with ADT, a subset of patients can develop a more aggressive, castration-resistant prostate cancer (CRPC). The clinical efficacy of the CYP17A1 inhibitor, abiraterone acetate, in the treatment of CRPC confirms that the disease remains hormonally driven. Quantification of the androgen metabolome in serum and tumor biopsies can inform one as to the metabolic pathway that has been activated in individuals with CRPC. To this end, a stable isotope dilution liquid chromatography electrospray ionization tandem mass spectrometry (SID-LC-ESI-MS/MS) method has been developed to detect ketosteroids as Girard T oximes and hydroxysteroids as picolinic

esters. Accuracy and precision values have been obtained for ketosteroid quantification and glucuronate and sulfate conjugates quantitated using enzymatic hydrolysis. We have applied our method to quantify serum androgen levels from patients enrolled in the Total Androgen Pathway Suppression (TAPS) Trial.

R3 Functional Basis of Cytochrome P450 Oxidoreductase (POR) Deficiency Caused by the Q153R and A287P Mutations

Yi Jin[1], Walter L. Miller[2]

[1]Department of Pharmacology, Center of Excellence in Environmental Toxicology, University of Pennsylvania Perelman School of Medicine, [2]Department of Pediatrics, University of California, San Francisco

POR is a 2-flavin protein that transfers electrons from NADPH via its FAD and FMN moieties to all microsomal cytochrome P450 enzymes, including steroidogenic and drug-metabolizing P450s. Defects in the POR gene can cause POR deficiency (PORD) with clinical manifestations such as skeletal malformations, genital anomalies, and disordered steroidogenesis. We examined two disease-causing POR variants, Q153R, which caused both loss-of-function and gain-of-function in P450 activities in vitro, and A287P, which caused mostly loss-of-function in P450 activities in vitro. Flavin content analysis revealed that A287P is deficient in FAD and FMN binding, whereas flavin binding was not significantly affected for the Q153R mutant. Externally added flavin partially restored the cytochrome c reductase activity of the A287P mutant, suggesting flavin therapy for this frequent form of PORD (found in 40% of patients of European ancestry). Transient kinetic dissection of the reaction of POR with NADPH and the reduction of cytochrome c by POR using stopped-flow techniques revealed defects in individual electron transfer steps mediated by Q153R and A287P. Q153R behaved similarly to the wild type POR in its reaction with NADPH, but was defective in electron donation to cytochrome c. In contrast, A287P showed an impaired ability to accept electrons from NADPH, but was capable of a fast FMN to cytochrome c electron transfer. Thus the differential effects of Q153R on various P450s may be due to differences in FMN to heme electron transfer with different P450 partners, whereas the reduced rates of P450s with A287P may be due to deficient flavin and the impaired reaction with NADPH.

Supported by P30-ES013508 pilot project (YJ)

R4 Neutralizing IL-4 Rescues Inflammation in Neonatal Islets and Prevents β-cell Failure in Adult IUGR Rats

Lane J Jaeckle Santos, Changhong Li, G. Scott Worthen, Rebecca Simmons

Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA

Intrauterine growth retardation (IUGR) is linked to the later development of type 2 diabetes (T2D). We have developed an animal model of IUGR, which leads to the development of T2D in adulthood. Inflammation is associated with T2D and is a critical component of metabolic syndrome in both human and experimental models, but it is unknown whether inflammation is causal or secondary to the abnormal metabolic state. We hypothesized that IUGR induces fetal inflammation in the pancreatic islet, which in turn leads to decreased islet vascularity and impaired insulin secretion.

Microarray analysis of fetal and PD14 IUGR islets showed marked changes in expression of genes regulating immune mediated inflammation, macrophage activation and angiogenesis. Histological examination of e19 and PD14 IUGR islets show decreased capillary density, and invasion by T-lymphocytes and macrophages. Levels of IL-2, IL-4 and IL-10 were significantly elevated in fetal islet lysates, consistent with Th2 immune response. By PD14, systemic levels of insulin, leptin and the pro-inflammatory cytokines mcp1 and RANTES, which recruit

Reproduction, Endocrinology, and Development (READ)

macrophages to sites of inflammation, were significantly elevated, demonstrating systemic inflammation and the beginning of metabolic syndrome. To determine whether Th2 inflammation is responsible for the abnormal ß-cell phenotype, animals received neutralizing IL-4 antibody treatment or vehicle at PD days 1-5. Neutralizing IL-4 treatment restored islet capillary density by PD14, reduced circulating insulin levels, and ameliorated systemic inflammation. At 15 weeks of age, IUGR animals treated with neutralizing IL-4 antibody showed completely normal insulin secretion in isolated perifused islets at 15 weeks.

Our results demonstrate that adult-onset diabetes secondary to IUGR is both preceded by and caused by fetal islet inflammation, resulting in immune cell invasion, inflammatory cytokine release, decreased islet vascularity and increased insulin resistance. Administration of neutralizing IL-4 antibodies at the neonatal stage suppresses inflammatory cytokine levels, normalizes islet vascularity, and permanently restores insulin sensitivity, demonstrating a novel role for Th2 immune responses in the induction and progression of T2D and metabolic syndrome. At the neonatal stage, inflammation and vascular changes are reversible, and may define an important developmental window for therapeutic intervention to prevent adult onset diabetes.

R5 Investigating the Estrogen and Androgen Metabolome in the Progression Towards Hormone-independent Breast Cancer

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Estradiol and its metabolites are the major drivers of hormone-responsive breast cancers. Inhibition of aromatase, the enzyme responsible for conversion of androgen precursors into estrogens, has proven effective in both the prevention and treatment of breast cancer. However, resistance arises frequently, with 10-15% of women developing resistance in the course of 10 years. There has been a lack of sensitive and specific methodology to track therapeutic response to aromatase inhibitors on an individual basis. Our lab has previously demonstrated highly sensitive LC-MS quantification of estrone, 16α-hydroxyesterone, 4-methoxyestrone, and 2-methoxyestrone from human serum using nano-flow liquid chromatography and pre-ionized derivatives. This methodology can be expanded to enable quantification of a comprehensive panel of estrogens and androgens from human biological samples. Here we have expanded the assay to include keto-steroids 2-hydroxy-estrone, 4-hydroxy-estrone, dehydroepiandrosterone (DHEA), androstenedione and testosterone. LC-MS quantification of a comprehensive panel of estrogen and androgen metabolites in women with breast cancer undergoing therapy with aromatase inhibitors will enable rapid and reliable assessment of therapeutic efficacy, and can potentially shed light on differential profiles of estrogen and androgen metabolites in women who present with resistance.

Lung and Airway Disease

L1 O_3 Inhalation Attenuates the Budesonide Effects on Allergic Airway Inflammation and SP-D Production in the Lung of Balb/c Mice

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Background: Our previous studies on airway responses to allergen or O_3 exposure showed that C57BL/6 mice were significantly more protected against developing inflammation and airway hyperresponsiveness than Balb/c mice that were relatively deficient in their ability to produce the immunoprotective surfactant protein D (SP-D) in their airways. Presence of SP-D in the lung is important to prevent constitutive activation of the pulmonary innate immune system. Glucocorticoid treatment can upregulate SP-D production. The aim of this study was to investigate whether the anti-inflammatory and SP-D inducing effects of glucocorticoid treatment are altered in allergen or O_3 exposed mice.

Methods: We studied BALB/c and C57BL/6 mice sensitized and challenged with Aspergillus fumigatus (Af), exposed to air or O_3 and treated with different doses of budesonide (0, 0.1, and 1 μM). Lung function and airway inflammation were assessed. BAL SP-D content was analyzed by Western blot and ELISA. Lung cellular composition was analyzed by FACS.

Results: Treatment with budesonide significantly reduced inflammatory changes and airway hyperresponsiveness in mice that received allergen sensitization and challenge alone. Budesonide inhibited allergic airway hyperresponsiveness to MCh in a dose-dependent manner. Budesonide also induced a protective increase in SP-D in Af sensitized and challenged BALB/c mice together with inhibition of the inflammatory cell numbers, macrophages and CD4 T cells in the lung. O_3 induced exacerbation of allergic inflammation 4 days after allergen challenge in Balb/c (but not C57BL/6) mice. This was characterized by enhanced airway eosinophilia, neutrophil influx and increased release of IL-6, TNF-alpha, eotaxin and CCL17 indicating activation of the innate immune system in the lung. O_3-induced exacerbation of the inflammatory changes was associated with an impaired expression of SP-D in the BAL. The effects of budesonide on inflammatory cells were attenuated in the O_3-exposed Balb/c mice. Importantly, the budesonide induced increase in SP-D production was abolished by O_3 exposure ($p<0.05$, $n=6$). Our results indicate that Balb/c mice are more susceptible for O_3-induced exacerbation of allergic airway inflammation than C57BL/6 mice. This susceptibility is associated with an impaired ability to express SP-D. Balb/c mice sensitized and challenged with Af are highly responsive to glucocorticoid treatment. However this responsiveness together with the protective induction of SP-D is abolished when the mice are also exposed to O_3.

Conclusions: We speculate that the beneficial action of glucocorticoids in allergic airway inflammation maybe mediated at least in part by an induction of SP-D production in the lung and that O_3 inhalation attenuates the glucocorticoid effects.

Lung and Airway Disease

L2 FOXP1/P4 Knockout Increases Reactivity of Murine Airways, Upregulates Neuropeptide Y (NPY), and Stimulation with NPY Increases Reactivity of Human and Murine Airways to Methacholine

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Rationale: FOXP1 and FOXP4 play important roles in regulating epithelial cell lineage determination and in epithelial regeneration in the lungs. We hypothesized that deficiency of FOXP1/P4 may also modulate airway responsiveness to contractile stimuli.

Methods: Lung resistance was measured in FOXP1/P4 knockout (P1/P4 KO) mice and wild type mice. mRNA was then isolated from both the large and small airways of the P1/P4 KO, and transcriptomics were examined. Interestingly, Neuropeptide Y (NPY) mRNA levels markedly increased in the KO mice. Accordingly, naïve Balb/c mice lung slices were treated overnight with NPY and examined for carbachol-induced bronchoconstriction. Additionally, human precision cut lung slices (PCLS) from normal healthy donors also were stimulated with NPY and agonist-induced bronchoconstriction assessed.

Results: Compared to wild type mice, FOXP1/P4 mice exhibit increased airway hyperreactivity to methacholine. Microarray analysis of the P1/P4 KO lungs showed a 40-fold increase in transcripts for NPY. In both naïve Balb/c mice and human PCLS, NPY increased the sensitivity of the airways to carbachol-induced bronchoconstriction.

Conclusions: These data suggest that knockout of FOXP1/P4 modulates not only cell lineage fate, but may also promote airway hyper-responsiveness. Given these data, further study defining the molecular mechanisms by which knockout of these transcription factors alters responsiveness of human airways may offer novel therapeutic targets for airways diseases.

L3 Mediator Release Following Rhinovirus Exposure Is Selectively Attenuated by a Kinase Inhibitor but Not a Steroid in Human Lung Slices

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Rationale: Viral-induced respiratory infections, a leading cause of asthma exacerbations, are primarily evoked by rhinovirus. Direct effects of virus on small airways of humans and approaches to attenuate viral-mediated exacerbations of airways disease remain therapeutic challenges. We hypothesized that RV16 induces mediator release from human small airways, which may be modulated by steroid treatment or kinase inhibition.

Methods: Human precision cut lung slices (PCLS) from normal healthy donors, each containing a small airway (≤ 2 mm), were stimulated ex vivo with increasing concentrations of RV16 (105-107 Pfu) for 48 hr, with culture supernatants assessed for mediator release. Slices were pre-incubated with the narrow spectrum kinase inhibitor RV1088 or dexamethasone for 1 hr prior to rhinovirus stimulation.

Results: Exposure of human small airways to RV16 induced IL-8, IL-6, and IP-10 secretion, with levels of all mediators markedly increasing. RV1088 decreased RV16-stimulated IL-8, IL-6, and IP-10 levels, whereas dexamethasone exhibited little effect on either mediator release. Qualitative cilia beat frequency was unchanged by treatment with any of the treatments.

Lung and Airway Disease

Conclusions: These data suggest that virus selectively increases chemokine and cytokine secretion shortly following viral exposure. Narrow spectrum kinase inhibition attenuates virus-induced IL-8, IL-6, and IP-10 release but steroid treatment has little to no effect. Given these data, kinase inhibitors may serve to modulate the inflammatory mediator profile upregulated following viral exposure.

L4 Formaldehyde-induced Airway Hyperresponsiveness: Role of Ca2+ Dynamics in Human Airway Smooth Muscle (HASM) Cells.

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Background: Asthma is a chronic airway disorder clinically characterized by airway spasms, resulting from hyper-responsive airway smooth muscle (ASM). Altered Ca^{2+} handling mechanisms, including Ca^{2+}-sensitization, can contribute to hyper-responsive ASM cell. Formaldehyde (FA) is an important indoor air pollutant with known roles in asthma exacerbation and airway hyperresponsiveness (AHR). The mechanisms involved in FA-induced AHR are not clearly understood.

Hypothesis: We hypothesized that FA induces AHR through enhancing the contractile response of ASM.

Methods: Precision-cut human lung slices (PCLS) or human ASM (HASM) cells obtained from healthy donors were exposed to vehicle (saline) or FA (0.2, 0.8 or 2 ppm) for 1 h and incubated for 24 h in fresh medium. In PCLS, the contractile response to carbamylcholine (Cch), ciliary beat frequency (CBF) and secretion of IL-6 and IL-8 were determined. In HASM cells, agonist-induced cellular Ca^{2+} response ($[Ca^{2+}]i$), IL-6 secretion and Rho-associated Kinase (ROCK) activities were determined.

Results: Exposure to 2.0 ppm FA enhanced Cch-induced contractile response in PCLS without significant effects on IL-6/IL-8 secretion. The ciliary beat frequency was reduced in FA-treated PCLS. In HASM cells, agonist-induced $[Ca^{2+}]i$ and IL-6 secretion were not significantly altered by exposure to FA. In HASM cells exposed to 0.2 ppm FA, ROCK activity was elevated compared to that of vehicle-treated cells.

Conclusions: FA enhances Cch-induced airway contractility in PCLS. Ca^{2+} sensitization through Rho-associated kinase and enhanced ASM cell shortening may be the potential mechanisms of FA-enhanced airway contractility.

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L5 The Receptor for Advanced Glycation Endproducts is Increased in Pulmonary kHypertension Due to Advanced Lung Disease

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Background: The Receptor for Advanced Glycation Endproducts (RAGE) is a widely expressed multi-ligand pattern recognition receptor that is implicated in the development of systemic vascular disease. Engagement of RAGE leads to sustained cellular dysfunction marked by increased NfκB activation, and reactive oxygen species generation. Although RAGE ligands have been implicated in the development of pulmonary arterial hypertension (PAH), the role of RAGE in the development of secondary pulmonary hypertension (PH) remains

Lung and Airway Disease

unknown. We therefore hypothesized that sRAGE would be elevated in patients undergoing lung transplantation with secondary pulmonary hypertension when compared with those without pulmonary hypertension, and that higher plasma levels would be associated with more severe PH.

Methods: We conducted a cross-sectional analysis of a multi-center prospective cohort of patients enrolled in the Lung Transplant Outcomes Group study who underwent lung transplantation at 10 centers between 2002 and 2010. Plasma sRAGE was measured preoperatively utilizing ELISA. PH was defined by WHO criteria as mean pulmonary artery pressure > 25mmHg at time of transplant. Wilcoxon rank sum test and logistic regression were used to compare sRAGE levels between groups. Spearman's rank correlation was used to examine associations of RAGE levels with disease severity.

Results: The study sample included 714 patients (289 with COPD and 236 with IPF). The mean age was 52 and 47% were female. Four hundred and twenty (59%) had PH at the time of transplantation. sRAGE levels were significantly higher in those with secondary PH than in those without (median 772 pg/ml, IQR 444-1563 vs. 667, IQR 362-1265, p <0.01 and correlated with increasing mean PA pressure (Spearman's p=0.09, p=0.02). There was a significant interaction with diagnosis in analysis of the two largest diagnosis groups (COPD and IPF, p = 0.06). In a stratified analysis of these two groups, increased sRAGE was associated with increased odds of PH in patients with COPD but not IPF (OR per SD of RAGE 2.00 in COPD, 95%CI 1.06-3.80, p=0.03; OR of 0.98 in IPF, 95%CI 0.69-1.40, p=0.92).

Conclusions: Plasma sRAGE levels are higher in patients with PH at the time of lung transplantation than those of patients without PH, and correlate with severity of PH. This association appears to be driven primarily by COPD. Future studies to elucidate the mechanisms by which RAGE mediates the development of pulmonary hypertension in patients with COPD are warranted.

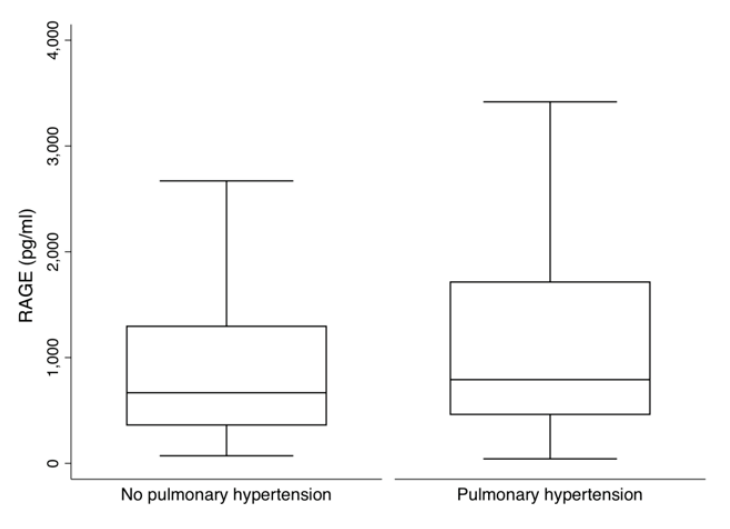

Figure 1: Box plot of RAGE values for patients with and without pulmonary hypertension.

Lung and Airway Disease

L6 Lack of Surfactant Protein A (SP-A) Enhances Airway Inflammation and Hyperresponsiveness After Ozone (O_3) or Aspergillus fumigatus (A*f*) Exposure in Association with Increased Presence of IL-13+/CD206+ Alternatively Activated (M2) Macrophages

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Background: M2 macrophages are important contributors to allergen-induced airway inflammation. The role of these cells in inflammatory responses not involving allergens such as O_3-induced lung injury is unclear. We previously showed that SP-A protects against allergic airway inflammation in mice. Here we hypothesize that presence of SP-A is needed to attenuate the inflammatory response to O_3 or Af inhalation by preventing abnormal M2 macrophage polarization in the lung.

Methods: SP-A-/- and wild type C57BL/6 mice were exposed to 3.0 ppm of O_3 for 2 hours and studied 0, 12, 24, and 48 hours later. Additional groups were sensitized i.p. (day 0 and 7), then intranasally challenged on day 13 with the fungal extract of Af and studied 48 hours later. Bronchoalveolar lavage (BAL) fluid cellular cytokine and SP-D content and lung function were compared between SP-A-/- and C57BL/6 mice. Multicolor FACS analysis was performed to study the composition of macrophage (M1 and M2) subsets and expression of IL-13, IL-10 and CCL17.

Results: When compared with controls, O_3 inhalation or a single A*f* challenge lead to significant increases in lung resistance and decreases in dynamic compliance with a peak 24 hours after O_3 exposure or 48 hours after A*f* challenge (n=7-8, $p<0.01$). This increase was enhanced in SP-A-/- mice that also had significantly higher number of neutrophils than C57BL/6 mice 24 hours (n=8, $p<0.05$) after O_3 exposure and of eosinophils 48 hours after Af challenge (n=7, $p<0.01$). O_3 exposure or A*f* challenge in both C57BL/6 and SP-A-/- mice induced significant TNF-α and IL-6 as well as IL-5 and IL-13 release into the BAL fluid during the peak of airway inflammation. On the other hand, increased SP-D release in response to O_3 or Af occurred concurrent with the resolution of inflammatory changes. SP-A-/- mice had heightened SP-D expression, higher numbers of CD4+T cells and F4/80+/CD206+ M2 macrophages (n=6-12, $p<0.05$) in the lung. SP-A-/- M2 macrophages expressed significantly greater intracellular IL-13 and CCL17, but lower IL-10, than C57BL/6 M2 macrophages.

Conclusions: Lack of SP-A in gene deficient mice resulted in enhanced airway inflammation and hyperresponsiveness after either O_3 or allergen exposure. In spite of a compensatory increase in SP-D levels in these animals, the amplified airway inflammation was associated with increased presence of pro-inflammatory M2 macrophages together with heightened TNF-α, IL-6, IL-13 and CCL17 expression. Thus, presence of SP-A is necessary to control airway inflammation in the lung elicited by environmental stimuli.

Gene-Environment Interactions

G1 Dietary Flaxseed Lignan Component (FLC) Administered Post Thoracic Radiation Exposure Decreases Inflammation-related Gene Expression Levels in Murine Lung

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Introduction: Flaxseed (FS) and its lignan component (FLC) are dietary supplements known for their anti-oxidant and anti-inflammatory properties. Rationale: Radiation exposure of lung tissues can occur either when given therapeutically to treat intrathoracic malignancies or when occurring incidentally such as in the case of exposure from inhaled radioisotopes released after the detonation of a radiological dispersion devise (RDD). Such exposure is associated with pulmonary inflammation, oxidative tissue damage and irreversible lung fibrosis. We have shown that dietary FS and FLC prevent pneumonopathy in a rodent model of thoracic X-ray radiation therapy (XRT). The gene expression changes in lung tissues, however, that are involved in regulating inflammation and help mitigate radiation effects post-exposure have never been evaluated.

Methods: We evaluated 10% FLC or isocaloric control diet given to mice (n=5/group) 24, 48, or 72 hours post a single dose 13.5 Gy thoracic XRT. Lungs were excised 24, 48 and 72 hours post-diet initiation, RNA extracted and quantitative reverse transcriptase polymerase chain reaction (qPCR) performed to determine gene expression changes related to inflammation. Data was normalized to beta actin and expressed as fold-change from non-irradiated control. Additionally, cytokine protein levels were evaluated in bronchoalveolar lavage (BAL) fluid from the same mice, using a 20-plex cytokine array.

Results: Radiation induced a robust increase of inflammatory gene expression level such as IL-6 detectable in lungs from control diet-fed mice at all time-points tested. Levels ranged from 2-5-fold over non-irradiated controls. Evaluation of lung tissues from 10% FLC-fed mice indicated a significant decrease of inflammatory gene expression most notably, IL-6 which remained at 1-1.6-fold over controls at all time points tested. Protein levels of cytokines in the BAL were detectable mostly in control-diet mice but remained low and did not increase significantly with radiation. Importantly, levels were beyond the lower detection limit of the assay for the BAL samples from FLC-fed mice.

Conclusions: Dietary FLC given post-irradiation mitigates radiation effects by decreasing expression of genes involved in inflammation. Dietary supplementation of FLC may thus, be a useful adjuvant treatment mitigating adverse effects of thoracic radiotherapy by increasing radiation tolerance of lung tissues in cancer patients or in individuals exposed to inhaled radioisotope.

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G2 Dietary Flaxseed Modulates the miRNA Profile in Irradiated and Non-irradiated Murine Lungs-A Novel Mechanism of Tissue Radioprotection by Flaxseed

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Introduction: As a dietary supplement, wholegrain flaxseed (FS), displays antioxidant and anti-inflammatory properties. We have previously shown that dietary FS protects against radiation pneumonopathy in a rodent model of thoracic X-ray radiation therapy (XRT). Specifically, FS enhanced survival and prevented adverse

Gene-Environment Interactions

radiation effects including pulmonary fibrosis, inflammation and oxidative lung damage when administered prior to radiation exposure and for the duration of the study (4 months).

Rationale: The mechanisms whereby dietary FS exerts radioprotective effects in lung are incompletely understood. MicroRNAs (miRNAs) are short oligonucleotides that act as important post-transcriptional regulators of inflammatory response networks and are also thought to participate in cell-to-cell communication. Responses of miRNA profiles to diet and to radiation exposure have been reported, but the potential contribution of miRNAs to diet-related radioprotection has never been tested.

Methods: Mice were fed 10% FS or a 0% FS isocaloric control diet for 3 weeks, a time needed for bioactive FS lignan metabolites to reach a steady level in the systemic circulation and exposed or not to a single-dose 13.5 Gy thoracic XRT. Mice were sacrificed 48 hours post XRT, RNA was extracted from lung tissue of three animals for each of the four experimental groups (+/- FS, +/- XRT), and small RNAs were profiled by OpenArray. Differential regulation of small RNAs was assessed by two-way ANOVA. Several individual two-group comparisons were also made.

Results: FS resulted in statistically significant expression differences for multiple miRNAs, including 7 with $p<0.001$. miR-150 was downregulated approximately 2.9-fold in the FS groups and is disproportionately integrated into immune response-related networks. Although few miRNAs were significantly changed by radiation exposure, interaction between diet and radiation was observed, e.g. for miR-29c, which was greatly downregulated in the FS control group (10 to 50-fold) but slightly upregulated in the FS radiation group. Compared with FS control, the FS/XRT group experienced a 50% decrease of the p53-responsive miR-34a, which regulates senescence- and apoptosis-related factors.

Conclusions: The induction by dietary FS of significant changes in lung miRNA profile suggests that i) modulation of small RNA by dietary supplements may represent a novel strategy to prevent the adverse effects of thoracic radiotherapy and ii) that this may be one of the mechanisms by which FS exerts its known radioprotective effects in lung. Our model provides a useful system to further explore and optimize such small RNA-based therapies.

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Community Outreach and Engagement Core

Resources for Education and Action for Community Health in Ambler ("REACH Ambler")

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Asbestos exposures in Ambler, PA began in the late 1800s when the Keasbey and Mattison Company began using asbestos to manufacture asbestos cement products. Asbestos-containing waste from the plant was dumped in several surrounding areas through the 1980s. These sites continue to present remediation challenges that the Environmental Protection Agency (EPA) is evaluating. Residents have many questions about how to understand the consequences of their exposure. The University of Pennsylvania's Center for Excellence in Environmental Toxicology (CEET) funded a pilot ethnographic study designed to identify community perceptions about environmental and occupational exposure to asbestos. Researchers identified several themes including significant uncertainty about risk and remediation mechanisms as well as the effects of asbestos exposure on community identity. The pilot study informed the team's successful application for a National Institutes of Health (NIH) Science Education Partnership Award (SEPA). The SEPA-funded study, "REACH Ambler," aims to 1) document the historical and current experience of living near and working at a contaminated site; 2) provide residents with data that can help shape the future of their community; 3) develop and evaluate a science education program that will elaborate on the history of asbestos manufacturing and resulting exposures in Ambler.

Biomarker

B1 Synthesis of Petrogenic PAH Metabolites For Analyte Identification

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The EHSCCs at UTMB and UPenn are involved in The Gulf Coast Health Alliance: health Risks related to the Macondo Spill (GC-HARMS)-U19-ES020676 which includes an assessment of the toxicology of petrogenic polycyclic aromatic hydrocarbons (PAHs) in the gulf-oil spill. Petrogenic PAH differ from the pyrogenic PAH (e.g benzo[a]pyrene) since they are heavily alkylated and oxygenated, and little information exists regarding their metabolism which may determine their toxicity. The parent grant has elucidated the metabolic profiles of 5-methyl chrysene [C1-chrysene] and 1-methyl-7-isopropyl phenanthrene [C4-phenanthrene(retene)] in human hepatoma (HepG2) cells using HPLC-UV/Fluorescence and LC-MS/MS. C1-Chrysene forms tetraols, mono- and bis-phenols, o-quinones and catechol-conjugates, indicative of P450 and AKR activation. By contrast C4-phenanthrene forms dihydrodiols, mono-phenols, o-quinones and catechol-conjugates indicative of AKR activation only. Since regio- and /or stereoisomers are possible for these metabolites authentic standards are required to validate analyte identity. Analyte identity could lead to rapid deployment of a biomarker assay to determine human exposure to petrogenic PAH. The Synthetic Core at UTMB is synthesizing a library of

Biomarker

authentic petrogenic PAH metabolites identified in HepG2 cells. The Translational Biomarker Core at UPenn will compare the LC-MS/MS properties of the authentic standards with those observed for the metabolites identified in HepG2 cells

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B2 Biomarkers of Pancreatic Cancer

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Identification of very early stage disease is currently the most promising approach that could reduce cancer mortality. In searching for biomarker for PDAC, serum was analyzed from two cohorts: early-stage resectable PDAC patients (n=20) and age-matched normal controls (n=20). The serum was extracted from each subject using a modified Folch extraction and analyzed by high-resolution nanospray UPLC-MS on the Thermo Scientific LTQ-Orbitrap-XL in positive ion nanospray ionization at a resolution of 100, 000. There were thousands of significantly different features in the global serum metabolomes between the two groups. Principal component analysis led to the identification of three sphingomyelins (SMs) and two phosphatidylcholines (PCs), which were shown in increased intensities in the PDAC patients' serum samples when compared with serum from normal controls. Quantitative analyses were conducted using a Thermo TSQ Quantum triple quadrupole mass spectrometer. Other metabolites that were clearly dysregulated were also determined.

Integrated Health Sciences Facility Core

Metabolism of Representative Alkylated and Oxygenated Petrogenic Polycyclic Aromatic Hydrocarbons in Human Hepatoma (HepG2) Cells

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Exposure to petrogenic polycyclic aromatic hydrocarbons (PAHs) in the food-chain is the major human health hazard associated with the Deepwater Horizon gulf-oil spill. Risk assessment is based on the assumption that petrogenic and pyrogenic PAHs have similar toxicological profiles yet petrogenic PAHs are either alkylated or oxygenated and information on their metabolism is lacking. We report the metabolic fate of 5-methylchrysene and retene as representative alkylated petrogenic PAHs, and 9,10-phenanthrenequinone as a representative oxygenated petrogenic PAH in human HepG2 cells. The structures of the metabolites were identified by HPLC-UV-fluorescence detection and LC-MS/MS. The identification of tetraols, ortho-quinones and O-sulfated catechols supports metabolic activation of 5-methylchrysene and retene by P450 and AKR isozymes. The identification of O-glucuronidated catechols and O-methylated-O-sulfated catechols supports metabolic detoxification of 9,10-phenanthrenequinone through termination of redox cycling by UGT, COMT and SULT isozymes.

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NOTES

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