Center of Excellence in Environmental Toxicology

Fifth Annual Symposium

The Environment, Reproduction, Endocrinology, and Development

May 20, 2011
Villanova Conference Center

Center of Excellence in Environmental Toxicology

FIFTH ANNUAL SYMPOSIUM

The Environment, Reproduction, Endocrinology and Development

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

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Fifth Annual CEET Symposium

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7:30 – 8:00 A.M. **BREAKFAST AND REGISTRATION**

MORNING THEME: EPIGENETICS OF REPRODUCTION

8:00 A.M. **Shelley Berger, PhD**
Epigenetics and Gametogenesis

8:30 A.M. **Ralph Meyer, PhD**
Environmental, Genetic and Epigenetic Basis of Male Infertility

9:00 A.M. **Richard Schultz, PhD**
ART Procedures and Epigenetic Regulation

9:30 A.M. **Folami Iderraabdullah, PhD**
Vinclozolin as an Endocrine Disrupting Chemical: Role in Epigenetic Imprinting
CEET Mentored Scientist Awardee

10:00 – 10:30 A.M. **COFFEE BREAK**

10:30 A.M. **Translation to the Communities: Endocrine Disrupting Chemicals and Community Environmental Health**
Edward Emmett, MD, MS and Others

11:00 A.M. Keynote Lecture 1: **Robert A. Hiatt, MD, PhD**
Director, The Bay Region Breast Cancer and Environment Research Center
Professor and Chair, Department of Epidemiology and Biostatistics
Director of Population Sciences and Deputy Director, UCSF Comprehensive Cancer Center

"An Epidemiologic Approach to the Environment, Early Development and Breast Cancer"

12:00 – 1:00 P.M. **LUNCH**

AFTERNOON THEME: PRE-TERM BIRTH AND DEVELOPMENTAL DEFECTS

1:00 P.M. **Rebecca Simmons, MD**
Intrauterine Growth-retardation and Epigenetics of Type-2 Diabetes

1:30 P.M. **Sam Parry, MD**
Proteome Biomarkers of Pre-term Birth

2:00 P.M. **Steve Whitehead, DPhil**
Folate Genotypes/Phenotypes and Neural Tube Birth Defects

2:30 P.M. **Jennifer Culhane, PhD**
The National Childrens' Study

3:00 – 4:15 P.M. **BREAK AND POSTERS**

4:15 P.M. Panel Discussion: **Challenges in Reproductive, Endocrine and Developmental Toxicology**

Sally Perreault Darney, PhD
National Health and Environmental Effects Laboratory, U.S. EPA

Kimberly Gray, PhD
Scientific Program Administrator, Community-based Participatory Research (CBPR), NIEHS

Elaine Z. Francis, PhD
National Program Director, Pesticides & Toxics Research Program, Office of Research & Development, U.S. EPA

5:00 P.M. Keynote Lecture 2: **Shuk-Mei Ho, PhD**
Professor of Epigenetics and Endocrinology
Jacob G. Schmidlapp Chair of the Department of Environmental Health, University of Cincinnati

"Environmental Epigenetics and Disease Risk"

6:00 P.M. **WINE AND CHEESE RECEPTION**

Keynote Speakers

Robert A. Hiatt, MD, PhD is the Director of Population Sciences and Deputy Director of the UCSF Helen Diller Family Comprehensive Cancer Center. He is a Professor and Chair of Epidemiology and Biostatistics at UCSF and also a Senior Scientist for the National Kaiser Permanente Medical Care Program in Oakland. Dr. Hiatt holds adjunct appointments as Professor in the Division of Epidemiology at the University of California Berkeley School of Public Health and the Division of Research at Kaiser Permanente Northern California in Oakland.

From 1998 to early 2003 he was the Deputy Director of the Division of Cancer Control and Population Sciences at the National Cancer Institute (NCI), where he oversaw cancer research in epidemiology and genetics, surveillance, and health services research. Before that he was the Director of Prevention Sciences at the Northern California Cancer Center and also Assistant Director for Epidemiology at the Division of Research, Kaiser Permanente Medical Care Program in Northern California.

He was trained in medicine at the University of Michigan and in epidemiology at the University of California Berkeley. He is Board Certified in Preventive Medicine and, until taking his NCI position, practiced general internal medicine. He is a past president of the American College of Epidemiology and the American Society for Preventive Oncology.

Principal Research Interests

After an early career in international health, in which he was interested in the impact of parasitic diseases on community health, he was primarily focused on cancer epidemiology, studies of cancer screening both in terms of efficacy and effectiveness in diverse community settings. In his position he took a broad national perspective on the agenda for cancer prevention and control research and initiated programs in large-scale studies of gene-environment interactions in epidemiology, centers for the study of population health and health disparities, and studies of the quality of cancer care. Now his research interests include cancer epidemiology especially breast cancer, cancer prevention and screening, and the social determinants of canter. His central focus at UCSF is building a strong, interdisplinary program in epidemiology and cancer population sciences that include genetics, behavior and health services research, surveilance, and survivorship research. He is PI of the Bay Area Breast Cancer and the Environment Research Center that is studying the influence of environmental factors on pubertal maturation as a window to understanding the causes of breast cancer.

Dr. Shuk-mei Ho is internationally recognized for her expertise in the role of hormones, endocrine disruptors (EDC), and their receptor signaling on disease development including tumorigenesis in the prostate, ovary, endometrium and breast. Unraveling the functions of estrogen receptor beta (ERb) in the prostate has been one of her key focuses over the past decade. She revealed complex signaling crosstalk between ERb and its protein isoforms during prostate cancer progression. She has also made significant contributions in the impact of heavy metals, oxidative stress and inflammation to carcinogenesis, discovery of biomarkers for cancer detection and patient classification, and mechanism-based drug development. Her research is pivotally anchored on modern investigative tools for genomics, transcriptomics, proteomics, epigenomics, and informatics research that focus on improving predictive, preventive medicine.

Dr. Ho's current research extends to developmental bases of disease susceptibility by applying epigenetics to epidemiological studies, addressing two of the important challenges of research in environmental exposure and human health — multiple exposures at various developmental stages and the trans-generational effects of exposure. Her recent findings on EDC exposure, including bisphenol A, raise concerns on in utero exposure to EDC in food and drinking water. She is also interested in elucidating how EDCs perturb ERb signaling pathway.

Past president of the Society for Basic Urological Research (SBUR), Dr. Ho is an active participant in the American Urologic Society, the Endocrine Society, the Society for Basic Urologic Research, Prostate Cancer Research Program, the Society of Toxicology, and the American Association for Cancer Research. She regularly chairs scientific review and policy committees for the National Institutes of Health and the Department of Defense. She is a member of the Integration Panel of the Department of Defense Congressionally Directed Prostate Cancer Research Program, and is on the Emerging Science Committee of the National Academy of Science. Dr. Ho was honored in 2007 by the Senate of the 127th General Assembly of Ohio, and received the Women in Urology Award from the SBUR and the Society of Women in Urology, primarily on her discovery of the adverse effects of early exposure to bisphenol A on prostate cancer risk. Dr. Ho also participated in a national review of key scientific literature linking environmental factors to female reproductive disorders. Collectively, her work has contributed to the recently passed "Toxic Toy Bill" in California and the re-examination of the risk of bisphenol A by the National Toxicology Program.

Dr. Ho has published over 164 papers in prestigious, peer-reviewed journals. She serves as the Director of the NIEHS funded Center for Environmental Genetics, and the Director of the Genomic Microarray Laboratory at the University of Cincinnati, College of Medicine.

MISSION

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 seventeen designated Environmental Health Science Core Centers in the nation.

The CEET mission is to understand the mechanistic link between environmental exposures and diseases of environmental etiology. Understanding these processes can lead to early diagnosis, intervention and prevention strategies. The end result will be to improve environmental health and medicine in our region.

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 to facilitate an integrative approach to environmental health/medicine. Although primarily housed in the School of Medicine, the fifty-five CEET Investigators belong to sixteen departments and five schools at the University of Pennsylvania.

The CEET marries its relevant research excellence to diseases of environmental etiology that affect our urban region. The CEET includes an affinity group in Lung and Airway Disease (asthma, lung cancer, mesothelioma, and chronic obstructive pulmonary disease) because of the poor air-quality and air-pollution in our region (ozone, fine particulate matter, allergens, SO_2, NO_2 and CO emissions). The CEET also has an affinity group in Endocrine and Reproduction Disruption because of the high incidence of adverse pregnancy outcomes that lead to low-weight birth and birth and developmental defects in our region. These organ-based cores are linked to our affinity groups in disease mechanism, which include Oxidative Stress and Oxidative Stress Injury and Gene- Environment Interactions.

The CEET facilitates research by supporting two large facility cores. The Molecular Profiling Core employs toxicogenomic, toxicoproteomic, biomarker and metabolomic approaches to conduct predictive molecular toxicology at a systems-wide level to identify molecular fingerprints of toxicant exposure and response, and disease of environmental etiology. The Integrative Health Sciences Facility Core provides the infrastructure to perform patient and population based environmental health research. It is equipped with the means to conduct human inhalation studies (inhalation chamber-planned opening Fall 2010), epidemiological studies in targeted communities, to access human biospecimens through a CEET virtual biorepository, and the means to conduct study design and biostatistical analysis on genetic and non-genetic projects.

The CEET aims to conduct research relevant to the forty-five Superfund Sites that permeate the region. Studies will elucidate: mechanisms of chemical toxicity; exposure levels, risk assessment and health hazard; bioremediation approaches; and effects on ecosystems and biodiversity.

The Community Outreach and Engagement Core (COEC) works with and disseminates research findings to select local communities to empower them with new knowledge so that they are better informed to tackle issues of health disparities and environmental justice. To improve the environmental health of these and similar affected communities, the COEC 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 COEC also disseminates its mission and its research 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.

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

Co-Leader: Ian Blair, PhD
Co-Leader: Harry Ischiropoulos, PhD
Paul Axelsen, MD
Joseph Baur, PhD
Michael Beers, MD
Jeffrey Field, PhD
Aron Fisher, MD
Garret FitzGerald, MD
Benoit Giasson, PhD
Toshinori Hoshi, 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

Co-Leader: George Gerton, PhD
Co-Leader: Samuel Parry, MD
Kurt Barnhart, MD, MSCE
Marisa Bartolomei, PhD
Shelley Berger, PhD
Samantha Butts, MD, MSCE
Ted Emmett, MD, MS
Brett Kaufman, PhD
Karen Knudsen, PhD
Jianghong Liu, PhD, RN
Ralph G. Meyer, PhD
Mary Mullins, PhD
Katherine Nathanson, MD
Trevor Penning, PhD
Richard Schultz, PhD
Rebecca Simmons, MD
Sindhu Srinivas, MD, MSCE
Wenchao Song, PhD
P. Jeremy Wang, MD, PhD

Affinity Group III
LUNG AND AIRWAY DISEASE

Co-Leader: Michael Beers, MD
Co-Leader: 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
James Kreindler, MD
Vera Krymskaya, PhD
Frank Leone, MD
Rey Panettieri, MD
Trevor Penning, PhD
Anil Vachani, MD

Affinity Group IV
GENE-ENVIRONMENT INTERACTIONS

Co-Leader: Tim Rebbeck, PhD
Co-Leader: Alexander S. Whitehead, DPhil
Marisa Bartolomei, PhD
Shelley Berger, PhD
Ian Blair, PhD
Michael Burczynski, PhD
Jinbo Chen, PhD
Vivian Cheung, PhD
Jason Christie, MD, MSCE
Hakon Hakonarson, MD, PhD
John Hogenesch, PhD
Todd Lamitina, PhD
Caryn Lerman, PhD
Hongzhe Li, PhD
Jennifer Pinto-Martin, PhD, MPH
Katherine Nathanson, MD
Trevor Penning, PhD
Sarah Tishkoff, PhD

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Toxicoproteomics
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Biomarker
Associate Director: Clementina Mesaros, Ph.D.

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Jennifer Pinto-Martin, PhD, MPH
Alexander S. Whitehead, PhD

Lung and Airway Disease

L1 Rhinovirus 16 (RV16) induces enhanced airway hyper-responsiveness and elicits differential mediator release in human small airways

Koziol-White, Cynthia J.[1], Philip R. Cooper[1], Wai-Ming Lee[2], James Gern[2], Angela Haczku[1], Reynold A. Panettieri[1]

[1]University of Pennsylvania, Philadelphia, PA; [2]University of Wisconsin at Madison, Madison, WI

Rationale: Viral-induced respiratory infections are a leading cause of exacerbations of asthma. Some characterization of responses of airways to rhinovirus has been performed, but direct effects of virus on small airways of humans remain poorly understood. Our hypothesis was to characterize RV16's ability to alter bronchoconstriction/bronchodilation and mediator release from human small airways.

Methods: Human lungs were inflated with 2% low melting temperature agarose, dissected, airways identified, and core samples prepared containing small airways. Slices were stimulated ex vivo with RV16 for 24 or 48 hr, with culture supernatants assessed for mediator release and slices examined for brochoconstriction/bronchodilation.

Results: Exposure of human small airways to RV16 induced bronchoconstriction 48 hr, but not 24 hr, following stimulation, but had no effect on bronchodilation at either time point. At both 24 and 48 hr following stimulation, IP-10 release was markedly increased as compared to no viral stimulation. IL-8 expression was modulated by RV16 exposure, but GM-CSF, IL-1b, and SP-D release were not.

Conclusions: These data suggest that structural cells may also play a role in modulating responses to viral exposure, both contractile and mediator elaboration. Given these data, future therapeutics for virus-induced asthma exacerbations may focus not on prevention of productive infection, but alterations in the responsiveness of the lungs.

L2 Chronic inflammation is associated with increased Resistin-Like Molecule (RELM)-beta expression in Surfactant Protein D (SP-D) knockout mice

Sharma, Satish K., Melane Fehrenbach, Blerina Ducka, Sonja Kierstein, Cynthia Koziol-White, Gary Wu, Angela Haczku

University of Pennsylvania, Philadelphia, PA

RELM-beta and SP-D play significant regulatory roles during resolution of the inflammatory airway response in mice. SP-D exerts immunoprotective function in airway inflammation, but the role of RELM-beta and whether a regulatory relationship exists between them is not clear. Wild type C57BL/6 (WT), SP-D-/- and RELM-beta-/- mice were sensitized and challenged with Aspergillus fumigatus (Af) and studied at baseline, and 0, 1, 7 and 10 days later, for Methacholine responsiveness, airway inflammatory cell influx, cytokine profile and procollagen peptide release in the airways. Multiple gene expression was determined by a real-time PCR based microarray. SP-D-/- mice had foamy macrophages with markedly increased TNF-alpha and IL-13 expression and RELM-beta. Peritoneal macrophages ex vivo from mice after RELM-beta injection expressed TNF-alpha. No difference was found between WT and RELM-beta-/- mice in terms of inflammatory cell influx, IL-4, IL-5, IL-13 levels or methacholine responsiveness. RELM-beta induction coincided with procollagen peptides, collagen 1, 3a1, 4a3 and 6a1 as well as HAPLN1, LAMA1 and Tgfb2 genes. The HAPLN1 and LAMA1 mRNA expression was significantly lower in RELM-beta-/- mice at day 7 and 10. Heightened expression of RELM-beta in SP-D-/- mice may contribute to maintenance of a proinflammatory innate immune phenotype. Presence of RELM-beta during the resolution phase of the inflammatory airway response is associated with expression of profibrotic genes.

Oxidative Stress and Oxidative Stress Injury

O1 Dietary flaxseed administered post-thoracic radiation treatment improves survival and mitigates radiation pneumonopathy in mice

Christofidou-Solomidou, Melpo[1], Sonia Tyagi[1], Kay See Tan[2], Floyd Dukes[1], Ralph Pietrofesa[1], Daniel F. Heitjan[2], Charalambos C. Solomides[4], Keith A. Cengel[3]

[1]Departments of Medicine, Pulmonary Allergy and Critical Care Division, University of Pennsylvania, Philadelphia, PA; [2]Department of Biostatistics & Epidemiology, University of Pennsylvania, Philadelphia, PA; [3]Department of Radiation Oncology, University of Pennsylvania, Philadelphia, PA; [4]Department of Pathology, Jefferson University Hospital, Philadelphia, PA

Background: Flaxseed (FS) is a known antioxidant and anti-inflammatory agent. Radiation exposure of lung can occur after exposure to inhaled radioisotopes released from a radiological dispersion devise (RDD) or an accident at a nuclear plant. Such exposure is associated with pulmonary inflammation, oxidative tissue damage and lung fibrosis. We reported that dietary FS prevents pneumonopathy in a rodent model of thoracic X-ray radiation (XRT). This study evaluated FS as a mitigator of radiation pneumonopathy.

Methods: We evaluated 10% FS or control diet given to mice (n=20–30 mice/group) on 0, 2, 4, 6 weeks post 13.5 Gy thoracic XRT vs. diet given preventively, at 3 weeks prior to XRT. Lungs were evaluated four months later for blood oxygenation levels, inflammation and fibrosis.

Results: Irradiated mice fed a 0% FS diet had a 4-month survival rate of 40% vs. 70–88% survival in FS-fed mice Additionally, all irradiated 10%FS-fed mice had decreased fibrosis vs. those fed 0%FS. Lung hydroxyproline content ranged from 96.5±7.1 to 110.2±7.7 μg/ml in irradiated, 10%FS mice vs.138±10.8 μg/ml for mice on 0%FS. Bronchoalveolar lavage (BAL) protein, Inflammation and weight loss was significantly decreased in all FS groups. All FS-fed mice maintained a higher blood oxygenation level vs. mice on 0% FS.

Conclusions: FS is a potent mitigator of radiation pneumonopathy.
Funded in part by: NIH-R01 CA133470-03, NIH-RC1AI081251-01, NIH-P30 CA016520.

O2 Effect of repair mutants on PAH-*o*-quinone induced mutations in p53

Louis-Juste, Melissa, Melissa Stengl, Zahidur Abedin, Sushmita Sen, Jeffrey Field

University of Pennsylvania School of Medicine, Department of Pharmacology, Philadelphia, PA

Polycyclic aromatic hydrocarbons (PAH) are ubiquitous environmental pollutants that are found in charbroiled foods, car exhaust and are a major carcinogen in cigarette smoke. Benzo[*a*]pyrene (B*a*P), a representative PAH can be metabolized into three mutagenic products: radical cations, anti diol epoxides, and PAH o-quinones. We have been studying BP 7, 8-dione (BpQ), a PAH *o*-quinone, which damages DNA via reactive oxygen species (ROS). We are using a yeast reporter assay to measure mutagenic properties of PAH metabolites. In this study we tested mutagenesis by BpQ in DNA repair mutants ogg1 (8oxoG-DNA glycosylase) and apn1 (Apurinic endonuclease). To determine the mutation rate, p53 cDNA was treated with BpQ and transformed into our yeast system. We did not find a significant difference between mutational frequency in wild type and apn1 yeast. However, mutation rates of ogg1 yeast were about 2-fold greater. Next, we isolated and sequenced mutant p53 plasmids to determine the mutational pattern and spectrum. In ogg1 cells, 70% of the mutations were G>T. In apn1 yeast, the predominant change was also G>T. Interestingly, we observed a strand bias in the apn1 yeast, where there were more G>T transversions in the non-transcribed strand compared to the transcribed strand. These data suggest that when base excision repair pathways are compromised, oxidative damage can cause a strand bias similar to that seen in smokers.

Oxidative Stress and Oxidative Stress Injury

O3 Cyclooxygenase-2-mediated formation of anti-proliferative oxidized lipids

Snyder, Nathaniel W., Xiaojing Liu, Suhon Zhang, Jasbir S. Arora, Sumit J. Shah, Ian A. Blair,

Center of Excellence in Environmental Toxicology, University of Pennsylvania, Philadelphia, PA

Cyclooxygenases (COX) can metabolize arachidonic acid (AA) to several oxidized lipids, called eicosanoids, including the hydroxyeicosatetraenoic acids (HETE). Many of these eicosanoids can then be further oxidized by dehydrogenases such as 15-prostaglandin dehydrogenase (15-PGDH). It is well-documented that different COX-2 derived eicosanoids exert cell type-specific effects on inflammatory response, cell growth and proliferation (pro- and anti-proliferative) as well as tumorigenesis. Interestingly, COX-2 is up-regulated in human cancers, whereas, 15-PGDH is down-regulated. Therefore the status of COX-2 and 15-PGDH levels in a cell may be of importance in the progression of cancer by altering the prevalence and fate of specific eicosanoids. The present study was aimed to identify novel COX-2-derived eicosanoids and to investigate their anti-proliferative activity.

Gene-Environment Interactions

G1 Effects of Bisphenol A Exposure on Genomic Imprinting in the Mouse

Susiarjo, Martha, Joanne Thorvaldsen, Christopher Krapp, Marisa Bartolomei

Department of Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA

Increasing data have suggested that environmental exposure during early development causes epigenetic reprogramming that lead to unfavorable health outcomes. Recent studies have demonstrated that some chemicals can alter DNA methylation, including the plastic compound bisphenol A (BPA) that is associated with health abnormalities in humans and model organisms. Our work focuses on effects of exposure on imprinted genes, genes that are epigenetically modified so that a single parental allele is expressed while the other is silenced. Appropriate imprinting is critical for normal growth and development and its misregulation is associated with diseases, including Beckwith-Wiedemann Syndrome (BWS) and Prader-Willi Syndrome (PWS). Our preliminary experiments have found that prenatal BPA exposure results in abnormal imprinting of genes linked to diseases, including Snrpn (PWS) and Igf2 (BWS). These effects were tissue-specific (embryo vs. extraembryonic tissue) and the changes at the Snrpn locus were DNA methylation-dependent. Our ongoing studies include analysis of more genes and of effects when lower doses are used. Additionally, we will undertake a more global approach by studying genome-wide DNA methylation and gene expression patterns through methylated DNA immunoprecipitation (MeDIP)-sequencing and microarrays, respectively. Results of our work may elucidate the potential etiology of diseases with underlying environmental causes.

G2 Comparative epigenomics approaches to study cell type specific gene regulation

Won, Kyoung Jae[1], Nha Nguyen[1], Heewoong Lim[1], Bing Ren [2], Wei Wang[2]

[1]University of Pennsylvania, Philadelphia, PA; [2]University of California, San Diego, CA

We also present a novel approach (called ChroModule) that classifies genomic region based on epigenomic information. Using supervised learning method on hidden Markov models (HMMs), we provide a new angle to see the epigenomic data with the HMM states. One of the most important advantages of the proposed method is that it can be further applied without retraining HMMs. The trained HMM in one cell line is applied to annotate genomic region in other cell types and used in capturing cell-type specific enhancers. Epiegenetically variant regions were more related with cell signaling or response. Cell type specific enhancers revealed cell functions as well as transcription factor enriched in enhancer. We also present a promoter/enhancer predictor. The predictor based on the wavelet transformation can identify regulatory elements without training procedure. Especially, the proposed algorithm can trace the epigenetic changes before and after the treatment.

Biomarker

B1 A Sensitive LC MS/MS Method for Absolute Quantitation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in Non-smokerÔøΩs Urine

Bhat, Showket H.[1], Clementina A. Mesaros[1], Anil Vachani[2], Ian A. Blair[1]

[1]Center of Excellence in Environmental Toxicology, University of Pennsylvania, Philadelphia, PA;
[2]Division of Pulmonary, Allergy and Critical Care, University of Pennsylvania, Philadelphia, PA

4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is a carcinogenic nitrosamine produced upon curing tobacco. It is present in tobacco smoke and undergoes metabolism to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in the lungs. NNAL has been shown to be a strong lung carcinogen in several animal studies and recently has emerged as a candidate biomarker for exposure to environmental tobacco smoke (ETS). The ability to conduct validated analyses of free and conjugated NNAL in human urine is important in order to assess inter-individual differences in lung cancer risk from exposure to cigarette smoke. Our study describes a novel derivatization procedure resulting in the formation of a pre-ionized NNAL derivative. The increased sensitivity arising from this derivative makes it possible to analyze free NNAL in only 0.25 mL urine by stable isotope dilution liquid chromatography electrospray ionization multiple reaction monitoring mass spectrometry (SID-LC-ESI -MRM/MS). *(We gratefully acknowledge support of grants UO1 ESO16004 and P30 ESO13508).*

B2 Structural characterization of the o-methylated-catechol metabolite of benzo[*a*]pyrene-7,8-dione in three human lung cells

Huang, Meng, Li Zhang, Ian A. Blair, Trevor M. Penning

Center of Excellence in Environmental Toxicology and Center for Cancer Pharmacology, University of Pennsylvania, Philadelphia, PA

Benzo[*a*]pyrene (B[*a*]P), a representative polycyclic aromatic hydrocarbon, is a ubiquitous environmental pollutant occurring in tobacco smoke and residues of fossil fuel combustion. Metabolic activation of the proximate carcinogen B[*a*]P-7,8-trans-dihydrodiol by aldo-keto reductases (AKRs) leads to B[*a*]P-7,8-dione that is redox-active and generates reactive oxygen species resulting in oxidative DNA damage in human lung cells. O-methylation of the corresponding catechol by cactechol-O-methyltransferase (COMT) is predicted as one pathway for detoxification of B[*a*]P-7,8-dione. We investigated the occurrence of this pathway in human lung adenocarcinoma A549 cells, human bronchoalveolar H358 cells and immortalized human bronchial epithelial HBEC-KT cells following treatment of B[*a*]P-7,8-dione for 24 hours. After acidification of the culture medium, a single o-methyl-B[*a*]P-7,8-catechol product was detected in the organic phase of medium from each cell line using HPLC-UV and LC-MS/MS. An authentic metabolite standard was subsequently produced by enzymatic synthesis and purified by semi-preparative HPLC and characterized by [1H]-NMR. The definitive structure of the cellular metabolite was identified to be o-8-methyl-B[*a*]P-7,8-catechol. It is concluded that human COMT may play a critical role in the detoxification of B[*a*]P-7,8-dione in lung cells *[Supported by P30-ES013508 and 1R01-CA-39504 awarded to TMP].*

Biomarker

B3 Quantitation of the benzo[*a*]pyrene (B[*a*]P) metabolome by a stable isotope dilution tandem mass spectrometry method and its application to human bronchoalveolar cells

Lu, Ding[1], Ronald Harvey[4], Ian Blair[2,3], Trevor Penning[1,3]

[1]Center for Excellence in Environmental Toxicology, University of Pennsylvania, Philadelphia, PA; [2]Center for Cancer Pharmacology, University of Pennsylvania, Philadelphia, PA; [3]Department of Pharmacology, University of Pennsylvania, Philadelphia, PA; [4]The Ben May Department of Cancer Research, University of Chicago, IL

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants and are carcinogenic in multiple organs and species. Recently, benzo[*a*]pyrene (B[*a*]P), a representative PAH has been designated as a Group 1 human carcinogen by the International Agency for Research on Cancer. B[*a*]P requires metabolic activation to exhibit its toxicity and carcinogenicity. The three major metabolic pathways involved include formation of radical cations (peroxidase mediated), diol-epoxides (P450 mediated) and o-quinones (aldo-keto reductase mediated). We want to determine the contributions of these different metabolic pathways to cancer induction and prevention by quantitating signature metabolites from each metabolic pathway. By successfully designing and synthesizing a library of [13C4]-labeled B[*a*]P metabolite internal standards, we developed a sensitive stable isotope dilution atmospheric pressure chemical ionization tandem mass spectrometry method to quantitate B[*a*]P metabolites. The LOD of the generally accepted biomarkers for B[*a*]P exposure, such as B[*a*]P-7,8-dihydrodiol and 3-OH-B[*a*]P, by this method was 1.5 fmol on column and their LOQ was 6 fmol on column. This method exhibits a 500 fold increased sensitivity compared with a method using HPLC-radiometric detection, which has a LOD of 1 pmol on column. This method was then applied to study the B[a]P metabolome in human bronchoalveolar H358 cells in the presence and absence of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a cytochrome P450 inducer. The sensitivity of the method should permit the metabolism of B[*a*]P to be measured in any setting and could also be used for biomonitoring human exposure to B[*a*]P. *[Supported by 1P30-ES013508 and 1R01-ES-15857 to TMP]*

B4 Detoxification of structurally diverse PAH *o*-quinones by human recombinant COMT via o-methylation of PAH catechols

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Polycyclic aromatic hydrocarbons (PAH) are ubiquitous environmental pollutants. Metabolic activation of intermediate PAH trans-dihydrodiols by aldo-keto reductases (AKRs) leads to o-quinones that are redox-active and carcinogenic to human lung cells. We investigated whether o-methylation by human recombinant COMT is feasible for the detoxification of a panel of structurally diverse PAH catechols produced during the redox-cycling process. Several classes of PAH *o*-quinones produced by AKRs were employed in the studies. PAH *o*-quinones were reduced to corresponding catechols by dithiothreitol under anaerobic conditions and then further o-methylated by human COMT in the presence of S-adenosyl-L-methionine as a methyl group donor. The formation of the o-methylated catechol was detected by HPLC-RAM-UV and LC-MS-MS. Human COMT was able to catalyze o-methylation of most of PAH catechols and generate isomeric metabolites. It is concluded that human COMT may play a critical role in the detoxification of PAH *o*-quinones *[Supported by 1R01-CA-39504 awarded to TMP].*

Reproduction, Endocrinology, and Development

R1 Structure activity relationship studies of *N*-phenylanthranilic acid based AKR1C3 inhibitors

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Aldo-keto reductase 1C3 (AKR1C3) has been implicated in the development of castrate resistant prostate cancer (CRPC). CRPC is associated with increased androgen receptor (AR) signaling brought about by elevated intratumoral androgen biosynthesis among other factors. AKR1C3 catalyzes the formation of testosterone and the proliferative prostaglandins F2alpha Inhibitors of AKR1C3 with little or no effect on the related isoforms, AKR1C1 and AKR1C2 are desirable in the context of CRPC as the latter enzymes are involved in the inactivation of 5α;-dihydrotestosterone. *N*-phenylanthranilates (*N*-PA) are non-selective AKR1C3 inhibitors. Using the flufenamic acid, as a lead compound, analogs representing modifications on the two aromatic rings were evaluated for AKR1C3 inhibitory activity. Structure activity relationship studies showed that the meta-carboxylic acid group was essential for AKR1C3 selectivity. Also, additional substitution on the A-ring generally led to similar or lower AKR1C3 inhibitory potency with no major effect on the selectivity. The 4' substituted 3-(phenylamino)benzoic acids were found to be the most potent and selective AKR1C3 inhibitors. These analogs displayed nanomolar affinity and significant selectivity for AKR1C3. These compounds are promising leads for drug development in CRPC and other malignancies with pathologic AKR1C3 activity. *[Supported in part by 1R01-CA90744 and a Challenge Grant from the Prostate Cancer Foundation, to TMP]*

R2 Development of stable isotope dilution liquid-chromatography mass spectrometry (LC/MS) methods for the determination of the androgen metabolom

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Prostate cancer is initially responsive to androgen ablation, but can recur as castrate resistant prostate cancer (CRPC). The success of the CYP17A1 inhibitor abiraterone acetate in the treatment of CRPC indicates that this disease remains hormonally driven. Determination of the androgen metabolome in patient samples would clarify which enzymatic steps contribute to androgen biosynthesis. Analysis of androgens in serum from patients undergoing treatment for prostate cancer could also provide an early predictor of efficacy and aid in tailoring personalized treatments. To this end, we developed stable isotope dilution LC/MS methods for determining androgens in serum. Derivatization of ketosteroids as Girard T oximes and hydroxysteroids as picolinic esters has been utilized, which improves ionization and introduces reliable mass transitions for every analyte. This strategy allowed detection of pg or lower quantities of all of the androgens. The derivatized steroids are separated with reverse phase HPLC and quantified through comparison to deuterated internal standards using electrospray ionization-MS detection. Using Girard T derivatization for ketoandrogens, we analyzed androgen levels in serum from patients undergoing prostate cancer treatment. Leuprolide significantly reduced levels of testosterone, but had limited effect on other androgens. Combination therapy with leuprolide and abiraterone acetate drastically reduced the levels of all of the androgens analyzed.

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R3 Crystal structure of human type 5 17beta-hydroxysteroid dehydrogenase (AKR1C3) in complex with 3-(4-(trifluoromethyl)phenylamino)benzoic acid

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Castration resistant prostate cancer (CRPC) is characterized with enhanced androgen receptor signaling. Over-expressed AKR1C3 in CRPC elevates intratumoral androgen level and is the culprit for tumor progression. An ideal AKR1C3 inhibitor should be selective towards the enzyme but impose minimal effects on the other AKR1C isoforms involved in androgen metabolism in the prostate. Based on the crystal structure of AKR1C3•$NADP^+$•flufenamic acid, we have synthesized and screened a family of N-phenylanthranilate AKR1C3 inhibitors. One of our lead compounds, 3-(4-(trifluoromethyl)phenylamino)benzoic acid (TFPB), has nanomolar affinity for AKR1C3 and exhibits greater than 250-fold selectivity for AKR1C3 over the other AKR1C isoforms, whereas flufenamic acid shows only a seven-fold selectivity between AKR1C3 and AKR1C2. Here we report the X-ray crystal structure of AKR1C3 in complex with NADP+ and TFPB (at 2.5 Åresolution) obtained by co-crystallization and determined by molecular replacement. TFPB is anchored to the oxyanion site through the carboxylate group and its trifluoromethyl substituted N-phenyl ring extends into the same binding subpocket as flufenamic acid. However, due to the meta-substitution in the benzoic acid ring, the N-phenyl ring is shifted and penetrates more deeply into the subpocket. The penetration is likely to prevent binding of TFPB to the other AKR1C isoforms and is the basis of the observed selectivity of this agent on AKR1C3 over the other AKR1C isoforms.

R4 Metabolism of the synthetic progestin norethynodrel by human ketosteroid reductases of the aldo-keto reductase superfamily

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Human ketosteroid reductases of the aldo-keto reductase (AKR) superfamily, i.e. AKR1C1-4 enzymes, are implicated in the biotransformation of synthetic steroid hormones. Synthetic progestin norethynodrel, which was the progestin component of the first marketed oral contraceptive, is known to undergo rapid metabolism to estrogenic 3alpha- and 3beta-hydroxy metabolites. The enzyme system(s) responsible for this transformation have not been identified. Norethynodrel is structurally similar to the hormone replacement therapeutic tibolone, with the difference being the presence of a 7-methyl group in the latter compound. We have previously shown that tibolone is bioactivated to 3alpha- and 3beta-hydroxy metabolites by human AKR1C enzymes. In this study, we show that AKR1C enzymes catalyze efficient reduction of 3-keto group of norethynodrel. Each individual enzyme displayed distinct kinetic property and stereochemical preference for 3alpha- or 3beta-hydroxy metabolite formation, which are similar to those with tibolone. Norethynodrel is also an isomer of norethindrone, with the difference being the position of the double bond. Norethindrone is the common progestin component in oral contraceptives and hormone replacement therapy and is not a substrate for AKR1C isofroms. Results suggest AKR1C enzymes are responsible for bioactivation of norethynodrel to its estrogenic metabolites in liver and target tissues.

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R5 Overexpression of AKR1C3 (type 5 17beta-hydroxysteroid dehydrogenase) in LNCaP cells as a model of androgen metabolism in castration-resistant prostate cancer

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Increased intratumoral androgen biosynthesis can contribute to castration resistant prostate cancer (CRPC). Upregulation of 17beta-hydroxysteroid dehydrogenase type 5 (AKR1C3), that converts 4-androstene-3,17-dione (A) to testosterone (T), has been detected in CRPC. To test whether AKR1C3 overexpression can contribute to CRPC by increasing T formation from adrenal androgens, we investigated [3H]-A metabolism in LNCaP and LNCaP-AKR1C3 (expressing AKR1C3) cells. In both cells, A was primarily reduced to 5alpha-androstane-3,17-dione and subsequently to (epi)androsterone. Observed levels of T were significantly higher in LNCaP-AKR1C3 cells. Addition of indomethacin, an AKR1C3 inhibitor, had a limited effect on A metabolism in LNCaP cells but reduced T levels in LNCaP-AKR1C3 cells to those of LNCaP cells. Addition of finasteride, a 5alpha-reductase inhibitor, eliminated formation of 5alpha-reduced metabolites. In LNCaP cells, T levels remained similar to control; in LNCaP-AKR1C3 cells, T levels markedly increased. Our findings indicate that AKR1C3 expression in a prostate cancer cell line can lead to significant production of T from A, which could increase androgen receptor (AR) activation and cancer growth. We also show that finasteride treatment increases T formation in LNCaP-AKR1C3 cells through redirection of the metabolic pathway and therefore may not be suitable to ablate AR activation in CRPC when AKR1C3 is overexpressed. *[Supported by 1R01-CA90744 awarded to TMP].*

NOTES

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