GEMS

Genetics and Environmental Mutagenesis Society

33rd Annual Fall Meeting

"The Impact of Environmental Exposures on Genomic Health Across Generations"

Wednesday, October 28, 2015

North Carolina Biotechnology Center
15 TW Alexander Dr.
Durham, NC 27703

Supported in part by grants from NIEHS.

NIH National Institute of Environmental Health Sciences

Contents

October 28, 2015

PRESIDENT'S MESSAGE

Dear GEMS Members and Guests,

Welcome to the 33rd Annual Fall Genetics and Environmental Mutagenesis Society Meeting. It has been a pleasure serving as the President of GEMS and I want to sincerely thank you for giving me the opportunity. I have enjoyed working with the Board of Directors (BOD) who dedicated their time for the betterment of this society.

Stephanie Smith-Roe, program chair, has put together an exciting program on "The Impact of Environmental Exposures on Genomic Health Across Generations". I am sure that we will leave the meeting with a better appreciation of how environmental exposures will impact the health of human populations across generations. Last spring meeting, which highlighted the role of genetic variation in shaping host responses to environmental exposures, was a great success. Highlights of this meeting included the use of systems genetics approaches in drosophila, mice, and genome-wide association studies in humans, to analyze quantitative traits. Stephanie did an outstanding job of lining up excellent speakers. I am sure Stephanie will be happy to sit back and run meetings, as the President, after organizing these two exceptional meetings.

Secondly, I would like to extend my heartfelt thank you to the GEMS Board of Directors (BOD) who have put in several hours of their time and were determined to keep the society strong and running. I particularly want to thank our secretary Holly Mortensen, for keeping the meeting minutes and maintaining an archive of GEMS records; as well as our treasurer Jef French, for monthly financial reports. Special thanks to William Ward, our webmaster who not only maintains an up-to-date website but also communicates with the members of the society. Other board members have equally contributed to planning monthly meetings and other societal activities. Thanks to all of them for their contributions as well.

Third, I want to thank our sponsors for their generous contributions which significantly helps the society in keeping up with the budget and serve the local scientific community. In particular I would like to thank Mr. Tom Hughes for his generous contribution which fostered the best presentation award for young investigators and helps the award winner to attend a national scientific meeting. I also would like to thank all the members of the GEMS society for their continuous support.

Finally, am happy to report that one of our previous GEMS speakers, Dr. Aziz Sancar from the University of North Carolina, is one of the recipients of this year's Nobel Prize in Chemistry, specifically for "mechanistic studies of DNA repair". This is an exciting advancement for a field that is paramount to the Genetic and Environmental Mutagenesis Society.

I am confident that I am leaving the society in exceptional hands and I wish everyone a wonderful Holiday Season and hope that you will enjoy the meeting.

Channa Keshava, Ph.D.
GEMS President

PRESIDENT ELECT (PROGRAM CHAIR'S) MESSAGE

Dear GEMS Members and Guests,

Welcome to the 33rd Annual Fall Meeting of the Genetics and Environmental Mutagenesis Society (GEMS). The theme of this meeting is "**The Impact of Environmental Exposures on Genomic Health Across Generations.**" Whereas much has been learned about the mechanisms of somatic cell mutagenesis, driven primarily by efforts to understand the process of carcinogenesis and to treat cancer, we are only beginning to gather detailed mechanistic data on how environmental exposures can affect the genetic code and how it is interpreted, and what this means for individuals and human populations with regard to adverse health outcomes.

David DeMarini, Ph.D., USEPA, has long asked the question, "Are there human germ cell mutagens?" and thanks to Dr. DeMarini, we may indeed soon have an answer[1]. Although research clearly supports the existence of rodent germ cell mutagens, extending that information to humans has been a considerable challenge, one that Dr. DeMarini will describe in his overview presentation.

The title of Dr. DeMarini's talk has put me into an interrogative frame of mind. Did you know that we arrived *Back to the Future* on October 21st, 2015? Scientists studying germ cell mutagenesis jumped into their DeLorean DMC-12 time machines and arrived back to the future a few years ago when advanced DNA sequencing technologies opened the door in a dramatic way to revisiting the effects of environmental exposures on the genomic integrity of the germ line[2]. **Carole Yauk, Ph.D.,** will extend the overview provided by Dr. DeMarini by describing the holistic advances made by Health Canada in the detection of germ cell mutations induced by a well-characterized environmental mutagen using Muta™Mouse as a model.

Did you also know that ~50% of the human genome isn't of human origin? Repetitive elements (such as retrotransposons) are being shown to have considerable influence over phenotypic differences among organisms[3]. **Rick Woychik, Ph.D.,** NIEHS, will discuss the important role of repetitive elements in epigenetic control of gene regulation and how these elements can be influenced by environmental exposures.

One last question. Did you know that your epigenetic slate was reprogrammed when you were haploid and again when you were an embryo[4]? **Folami Ideraabdullah, Ph.D.,** UNC-Chapel Hill, will describe for us how these waves of epigenetic reprograming are windows of susceptibility to the effects of essential nutrients and endocrine disrupting compounds.

A major part of the mission of GEMS is to provide a forum for graduate students, postdoctoral fellows, and staff scientists to hone their presentation skills and network with area scientists who share a common concern about genomic health, across a variety of disciplines from basic research to risk assessment. Let's give special thanks to the early career scientists who have volunteered to share their work with GEMS at this meeting.

Stephanie L. Smith-Roe, Ph.D.
GEMS President-Elect

[1]Demarini DM. (2013) Declaring the existence of human germ-cell mutagens. *Environ Mol Mutagen*, 53(3):166-72.
[2]Yauk *et al*. (2013) Harnessing genomics to identify environmental determinants of heritable disease. *Mutat Res*, 752(1):6-9.
[3]Ekram *et al*. (2012) Retrotransposons as a major source of epigenetic variations in the mammalian genome. *Epigenetics*, 7(4):370-82.
[4]Ideraabdullah et al. (2008) Genomic imprinting mechanisms in mammals. *Mutat Res*, 647(1-2):77-85.

GEMS
Genetics and Environmental Mutagenesis Society

The Impact of Environmental Exposures on Genomic Health Across Generations

8:00 am – 8:45 am	Registration and Continental Breakfast
8:45 am – 9:00 am	Welcome: **Channa Keshava**, Ph.D., GEMS President **Stephanie Smith-Roe**, Ph.D.,GEMS President-Elect
9:00 am – 9:30 am	**Are There Human Germ-Cell Mutagens? We May Know Soon** ***David M. DeMarini**, Ph.D., U.S. EPA, RTP, NC*
9:30 am – 10:30 am	**The Future of the Future: Next-Generation Analyses of Germ Cells to Protect the Next Generation** ***Carole L. Yauk**, Ph.D., Health Canada, Ottawa, ON, Canada*
10:30 am – 11:45 am	Poster Session and Sponsor exhibits
11:45 am – 12:45 pm	Lunch provided with registration
12:45 pm – 1:30 pm	**The Broad Impact of Repetitive Elements on Protein Coding Gene Expression in the Brain and Other Tissues** ***Richard P. Woychik**, Ph.D., NIEHS, RTP, NC*
1:30 pm – 2:30 pm	Talk by Postdoctoral Scholars and Graduate Students 1:30 – 1:45 – talk 1: Jenna Currier, Ph.D., U.S. EPA, RTP, NC 1:45 – 2:00 – talk 2: Dan Su, Ph.D., NIEHS, RTP, NC 2:00 – 2:15 – talk 3: Ma Wan, Ph.D., NIEHS, RTP, NC 2:15 – 2:30 – talk 4: Jose Zavala, Ph.D., U.S. EPA, RTP, NC
2:30 pm – 3:00 pm	Break (beverages and snacks)
3:00 pm – 3:45 pm	**Dissecting Mechanisms of Epigenetic Inheritance Using Mouse Models** ***Folami Y. Ideraabdullah**, Ph.D., Dept. of Genetics at UNC-Chapel Hill and UNC Nutrition Research Institute*
3:45 pm – 4:30 pm	Business meeting, Announcement of Awards etc.
4:30 pm	Adjourn

Genetics and Environmental Mutagenesis Society
Fall Meeting 2015
October 28th, NC Biotech Center (RTP)

The Impact of Environmental Exposures on Genomic Health Across Generations

Are There Human Germ-Cell Mutagens? We May Know Soon

David M. DeMarini, Ph.D.
EPA and Adjunct Professor, Dept. of Environment Sciences & Engineering, UNC-Chapel Hill

The Future of the Future: Next-Generation Analyses of Germ Cells to Protect the Next Generation

Carole L. Yauk, Ph.D.
Health Canada and Adjunct Professor, Dept. of Biology, Carleton University

The Broad Impact of Repetitive Elements on Protein Coding Gene Expression in the Brain and Other Tissues

Richard P. Woychik, Ph.D.
Deputy Director, NIEHS and Principal Investigator

Dissecting Mechanisms of Epigenetic Inheritance Using Mouse Models

Folami Y. Ideraabdullah, Ph.D.
Assistant Professor, Dept. of Genetics, UNC Nutrition Research Institute

Poster Session and Short Talks by Graduate Students and Postdoctoral Fellows
Awards for Best Talk and Poster Presentations

GEMS

http://www.gems-nc.org

Invited Speakers

David M. DeMarini, Ph.D., Integrated Systems Toxicology Division, U.S. Environmental Protection Agency, Research Triangle Park, NC, demarini.david@epa.gov

Biosketch: David M. DeMarini was born in Peoria, Illinois, USA on May 20, 1950. He received the B.S. (1972), M.S. (1974), and Ph.D. (1980) in Biological Sciences (genetics) at Illinois State University, Normal, IL. From 1980-1982, he did postdoctoral research at the Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN. He then was a Research Geneticist at the National Toxicology Program, National Institute of Environmental Health Sciences (NIEHS), RTP, NC from 1983-1984. He began his current position as a Genetic Toxicologist at the US Environmental Protection Agency, RTP, NC in 1985. He is also an Adjunct Professor, Dept. of Environ. Sci. & Engineering, School of Public Health, University of North Carolina, Chapel Hill, NC (1991-present). He is past-president of the Environmental Mutagenesis and Genomics Society (EMGS) and the International EMGS. He is an Editor of *Mutation Research--Reviews* (1998-present; impact factor 7.3). He has organized conferences, symposia, and training courses internationally, and has given invited lectures at more than 130 conferences in 55 countries. He has served on 8 WHO/IARC Monographs, chairing one of them. He has published >170 articles and has mentored 20 graduate students and postdocs. His research interests are molecular mechanisms of mutagenesis, mutation spectra, complex mixtures, and biomarkers of mutation in humans.

Abstract: Are There Human Germ-Cell Mutagens? We May Know Soon

The existence of agents that can induce germ-cell mutations in experimental systems has been recognized since 1927 with the discovery of the ability of X-rays to induce such mutations in *Drosophila*. Since then, various rodent-based assays have been used to identify ~50 germ-cell mutagens, as summarized in a workshop organized by the Environmental Mutagenesis and Genomics Society (EMGS) and the Jackson Laboratory (Wyrobek et al., EMM 48:71, 2007). Although no agent has been declared a germ-cell mutagen in humans, I used criteria similar to those of IARC for evaluating human carcinogens and identified four classes of agents as human germ-cell mutagens: chemotherapy, ionizing radiation, cigarette smoke, and urban air pollution (Environ Mol Mutagen 53:166, 2012). Consequently, the EMGS organized a working group called ENvironmentally Induced Germline Mutation Analysis (ENIGMA) to encourage the application of new genomic technologies to assess environmental influences on inherited diseases (Yauk et al., Mutat Res 752:6, 2013). The OECD has begun developing a series of Adverse Outcome Pathways (AOPs) on germ-cell mutagenicity to identify new technologies to assess germ-cell mutation that can fit into the rapidly evolving paradigm for toxicity testing (Yauk et al., Mutat Res 54:79, 2013; Yauk et al., Mutat Res, in press, 2015). A recent International Workshop on Genotoxicity Testing (IWGT) addressed current data gaps in the field, identified tools to fill these gaps, and proposed new models for when and how germ-cell mutagenicity testing should be conducted (Yauk et al., Mutat Res 783:36, 2015). In November

2017, IARC will host a scientific meeting to evaluate several agents as human germ-cell mutagens, presenting the possibility that an international organization may finally declare the existence of human germ-cell mutagens 90 years after the discovery of the first germ-cell mutagen. [Abstract does not necessarily reflect the views or policies of the U.S. EPA.]

Carole L. Yauk, Ph.D., Research Scientist, Health Canada, Adjunct Professor, Carleton University, Associate Editor, *Environmental and Molecular Mutagenesis,* Carole.Yauk@hc-sc.gc.ca

Biosketch: Carole L. Yauk obtained her B.Sc. (1993) and Ph.D. (1999) in biology from McMaster University in Hamilton, Ontario, Canada. Her Ph.D. thesis explored the effects of industrial air pollution on germline mutations in herring gull families. She joined the laboratories of Professors Sir Alec Jeffreys and Yuri Dubrova at the University of Leicester in England from 1999-2002 as a post-doctoral fellow. There she developed new single-molecule techniques to study induced mutations and meiotic recombination in mouse sperm. She returned to Canada to become a research scientist at Health Canada in 2002. She is currently lead of the Genomics Laboratory in the Healthy Environments and Consumer Safety Branch, and an adjunct professor of Biology at Carleton University. She is the founder and chair of Health Canada's Genomics Working Group. She won Health Canada's award for 'Most Promising Scientist' in 2006 and the Award of Excellence in Leadership in 2013. Dr. Yauk is Associate Editor of the journal *Environmental and Molecular Mutagenesis* and is on the editorial board of *Mutation Research.* She is a Canadian delegate to the OECD's Extended Advisory Group on Molecular Screening and Toxicogenomics and has taken on leadership roles in their Adverse Outcome Pathway program. She has been actively involved in the Environmental Mutagenesis and Genomics Society, having co-chaired special interest groups, many symposia and workshops, and having served as an elected councilor. Dr. Yauk recently co-chaired an IWGT workshop (Brazil, November, 2013) with the goal of advancing genetic toxicology testing for heritable genetic effects. Her current research is focused on the development and implementation of genomic approaches to improve chemical risk assessment, and on improving regulatory assessment of heritable effects. She has over 100 publications in these research fields.

Abstract: The Future of the Future: Next-Generation Analysis of Germ Cells to Protect the Next Generation

Carole L. Yauk, Ph.D. and Francesco Marchetti, Ph.D., Mechanistic Studies Division, Environmental Health Science and Research Bureau, Health Canada, Ottawa, ON, Canada

Whole genome sequencing is increasingly being applied in the clinic to identify *de novo* mutations that contribute to a wide range of human diseases. Although strong evidence supports that paternal age is positively correlated with increased offspring mutation burden, the role of environmental exposures is unclear. Our work aims to address this gap through the development of next generation approaches to measure germ cell and heritable mutations. We developed a protocol to evaluate chemically induced mutations across phases of spermatogenesis using

transgenic rodent mutation reporter models. Our studies apply the Muta™Mouse model, which contains a *lacZ* transgenic reporter gene that enables analysis of mutation frequency in any tissue. To compare new approaches in this field, we used the Muta™Mouse model to simultaneously study gene (*lacZ*) and microsatellite mutations in the sperm of mice exposed to the widespread environmental mutagen benzo[a]pyrene (BaP). Prior to euthanasia, male mice were mated with unexposed females to measure whole-genome copy number variants and point mutations in their offspring. Our data clearly indicate that BaP causes gene and microsatellite mutations in pre-meiotic male germ cells, including stem cells, confirming that it is a mouse male germ cell mutagen; BaP is most mutagenic to dividing spermatogonia. High-throughput sequencing of *lacZ* mutant plaques indicates slight differences in both spontaneous and BaP-induced mutation spectrum in sperm compared with bone marrow, suggesting differences in either the types of lesions induced in these different tissues, or in DNA replication/repair. Preliminary data indicate no effects on the induction of inherited copy number variants. Whole genome sequencing is currently being applied to pedigrees to assess the sensitivity of this tool for identification of germ cell mutagens. In a separate experiment, analysis of male mice exposed *in utero* to BaP revealed enhanced mutagenesis in both somatic and germ cells relative to exposed adults. These mice were exposed over a shorter period to lower doses of BaP than the adult exposures. The findings suggest that *in utero* exposure to environmental mutagens may contribute to increased mosaicism, which is emerging as important in a variety of human diseases. *In utero* exposure is thus a highly sensitive developmental window that should be more specifically studied in regulatory genetic toxicology. Overall, our group is working internationally with a variety of agencies and programs towards the long-term objective of developing a stronger regulatory paradigm to identify and assess germ cell mutagens.

Richard P. Woychik, Ph.D., Deputy Director, NIEHS and Principal Investigator, Research Triangle Park, NC, rick.woychik@nih.gov

Biosketch: Richard P. Woychik, Ph.D., is Deputy Director of the National Institute of Environmental Health Sciences, NIEHS. He is a molecular geneticist with a Ph.D. in molecular biology from Case Western Reserve University and postdoctoral training with Dr. Philip Leder at Harvard Medical School. He spent almost 10 years at Oak Ridge National Laboratory rising in the ranks to become head of the Mammalian Genetics Section and then director of the Office of Functional Genomics. In August 1997, Dr. Woychik assumed the role of vice chairman for research and professor in the Department of Pediatrics at Case Western Reserve University. In 1998, he moved to the San Francisco Bay area, first as the head of the Parke-Davis Laboratory for Molecular Genetics and then as chief scientific officer at Lynx Therapeutics. He returned to academics as the president and CEO of The Jackson Laboratory in August 2002 and served in that role until January 2011. Presently, Dr. Woychik is also a Principal Investigator in the Division of Intramural Research at NIEHS and is the head of the Mammalian Genome Group within the Epigenetics and Stem Cell Biology Laboratory.

Abstract: The Broad Impact of Repetitive Elements on Protein Coding Gene Expression in the Brain and Other Tissues

Tianyuan Wang[1#], Jian Feng[2#], Janine H. Santos[1#], Michael Cahill[2], David C. Fargo[1], Li Shen[2], Eric J. Nestler[2*] and Richard P. Woychik[1*] # These authors contributed equally to this work.

[1]National Institute of Environmental Health Sciences (NIEHS), 111 TW Alexander Drive, Building 101, Research Triangle Park, NC 27709; [2]Fishberg Department of Neuroscience and Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, Box 1065, New York, NY 10029.

Repetitive elements (REs) encompass 40-60% of mammalian genomic DNA and can influence the expression of adjacent genes. For instance, we previously showed that an intracisternal A particle (IAP) forms a fusion transcript with the adjacent *agouti* gene causing an obesity/type 2 diabetes phenotype in mice. To evaluate how often REs influence adjacent genes in a similar manner, we analyzed RNAseq data collected from the nucleus accumbens (NAc) in mice treated with cocaine. Here we demonstrate that 466 different genes express fusion transcripts that connect a RE with an adjacent protein coding exon through splicing. Most of these fusion transcripts are not influenced by cocaine. However, about 40% are either up- or down-regulated in response to this drug of abuse. Some of these fusion transcripts were expressed in a complex developmental and tissue-specific manner and vastly exceeded levels of their wild-type counterparts. Most notably, we demonstrate that the ectopic expression of a fusion transcript from the rho-guanine nucleotide exchange factor 10 (*Arhgef10*) substantially alters cocaine-reward behavior *in vivo*, which, like *agouti*, connects a fusion transcript to a phenotype in mice. Collectively these data indicate that REs impact many genes in a way that can influence the biology of the animal. *This research was supported in part by the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences, and NIDA.*

Folami Y. Ideraabdullah, Ph.D., Assistant Professor, Departments of Genetics and Nutrition, UNC Nutrition Research Institute in Kannapolis, University of North Carolina at Chapel Hill, folami@email.unc.edu

Biosketch: Folami Y. Ideraabdullah, Ph.D., is an Assistant Professor in the Genetics and Nutrition Departments at UNC Chapel Hill and a member of the UNC Nutrition Research Institute in Kannapolis. Her research program uses mouse models to identify and characterize DNA sequence differences that influence heritable epigenetic changes caused by environment. Current projects use a combination of nutrition models (ie. folate, choline, vitamin D, etc.) and endocrine disrupting models (vinclozolin and arsenic). The primary goal of these studies is to elucidate gene-environment interactions that influence susceptibility or resistance to environmentally-induced epigenetic perturbation and the role these factors play in determining heritability of both normal and disease related epigenetic states. This research has important implications in prevention, diagnosis, and treatment of diseases related to environmental exposures.

Abstract: Dissecting Mechanisms of Epigenetic Inheritance Using Mouse Models

Kiristin Clement [a], Ryan Kuster [a], Folami Ideraabdullah[a,b,c]

[a]University of North Carolina at Chapel Hill Nutrition Research Institute, 500 Laureate Way, Kannapolis, NC 28081; University of North Carolina at Chapel Hill, School of Medicine, [b]Department of Genetics, [c]Department of Nutrition, 120 Mason Farm Rd, Chapel Hill, NC 27599, United States.

A growing list of environmental factors are shown to perturb epigenetic states. These include naturally occurring and man-made compounds as well as dietary substances. Environmental perturbation of epigenetic states is recognized as a major contributing factor in disease mechanisms and its role in heritable disease phenotypes is of significant concern. Genomic imprinting is an epigenetic phenomenon characterized by monoallelic parent of origin dependent expression of a gene. More than a hundred imprinted genes have been identified in mammals with important roles in fetal and postnatal development, and significant contributions to diseases such as obesity, cancer and metabolic dysregulation. Here, we use a combination of environmental and genetic mouse models to investigate environmental perturbation of epigenetically regulated imprinted loci. Our findings show that perturbation of imprinted methylation marks is locus and tissue specific and that cis-acting mutations play an important role. I will discuss the implications of our findings in terms of mechanisms of epigenetic inheritance and the role of genetic makeup in determining the extent of epigenetic change and potential for heritability.

GEMS Lifetime Achievement Awards

"In Recognition of Outstanding Scientific Contributions and Dedication to GEMS"

This year, the GEMS Board of Directors wishes to recognize the extraordinary commitment to GEMS demonstrated by two founding members, Tom Hughes and David DeMarini.

Thomas J. Hughes, M.S., QA and Records Manager and Consultant, U.S. Environmental Protection Agency, Research Triangle Park, NC, Hughes.thomas@Epa.gov

Biosketch: Tom Hughes received his B.S. in Biology with a minor in Chemistry from Iona College in New Rochelle, NY in 1970, and a M.S. in Microbiology with a minor in Marine Science from N.C.S.U. in 1972 where he studied the degradation of crude oil and the metabolism of hydrocarbons by bacteria isolated from soil under Dr. Jerome Perry.

From 1972-1975 he was the lead scientist in the tissue culture laboratory of Dr. Melvyn Lieberman in the Physiology Department of Duke University where he studied the effect of cardiac drugs on chicken heart cells in culture. In 1975, he established the Ames/Salmonella laboratory at RTI, where he worked until 1990. During that time he conducted studies for the USEPA, NCI, the State of NJ and Industry. He helped established the GEMS in 1983 with a group of RTP scientists. GEMS is a local toxicology society which still holds two annual meetings today and offers beginning scientists the opportunity to present their research.

From 1990-1993, Mr. Hughes established an industrial Ames/Salmonella laboratory at EHRT in the RTP, NC, where he conducted studies for the NTP as the Lead Industrial Toxicologist. From 1993-1995, he was a Private Consultant. In 1995, he joined the NHEERL, USEPA, where for five years he conducted laboratory studies with Dr. Larry Claxton in the Ames/Salmonella assay. In 1999, he changed career direction and became a QA and Records Manager for Dr. Linda Birnbaum (who is now head of the NIEHS in the RTP, NC) at the USEPA. In 2009, he became the QA and Records Manager for the newly organized Research Cores for Dr. Russell Owen at the USEPA in the RTP, NC.

Mr. Hughes spent 25 years in the laboratory and 18 years as a QA and Records Manager and Consultant. He was fortunate enough to be able to use his skills in the laboratory to further his career as a QA and Records Manager at the USEPA. Along the way, he received the USEPA QA Manager of the Year Award in 2002 for his work with the World Trade Center dust. In 2015, he received a USEPA Gold Metal for his work with the Penobscot Indian Nation in Maine on studies with the Penobscot River water and sediment, drinking water, fish and other edible wildlife and plants that were conducted by a team of 50 scientists over a seven year period.

Mr. Hughes was one of the lead organizers of GEMS in 1982 and he has held every office except Secretary since then. He has served on the BOD several times and had been the President three times, the Corporate Sponsor Coordinator and the Secretary. The one thing he is proudest of during the 33 years GEMS has been in existence is all the scientists the Society has helped over these many years. That is why GEMS was formed.

Thomas will retire from the USEPA in October 2016. He thanks all of his colleagues and mentors who he has worked with and who have helped him over his career. It has been great fun and his extreme pleasure to work with each and every one of you! God bless you all.

David M. DeMarini, Ph.D., Integrated Systems Toxicology Division, U.S. Environmental Protection Agency, Research Triangle Park, NC, demarini.david@epa.gov

Biosketch: David M. DeMarini was born in Peoria, Illinois, USA on May 20, 1950. He received the B.S. (1972), M.S. (1974), and Ph.D. (1980) in Biological Sciences (genetics) at Illinois State University, Normal, IL, studying under Dr. Herman E. Brockman. From 1980-1982, he did postdoctoral research at the Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN. He then was a Research Geneticist at the National Toxicology Program, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC from 1983-1984.

He began his current position as a Genetic Toxicologist at the US Environmental Protection Agency (US EPA), Research Triangle Park, NC in 1985. He is also an Adjunct Professor, Dept. of Environ. Sci. & Engineering, School of Public Health, University of North Carolina, Chapel Hill, NC (1991-present). He is a member of the Environmental Mutagenesis and Genomics Society (EMGS) and the Genetics and Environmental Mutagenesis Society (GEMS). He has served as President of EMGS, GEMS, and of the International Association of Environmental Mutagenesis and Genomics Societies. He is an Editor of *Mutation Research--Reviews* (1998-present) and is on the Editorial Board of *Environmental and Molecular Mutagenesis* (1984-1989, 1993-present) and *Genes and Environment* (2006-present).

He has organized conferences, symposia, and training courses internationally, and has given invited lectures at more than 140 conferences in 55 countries. He has served on both (1986 and 2004) Tobacco Smoking and Cancer Monographs of the International Association for Research on Cancer (IARC) with WHO in Lyon, France, as well as the IARC Monographs on Drinking Water/Arsenic, Indoor Air, Vol. 100 Human Carcinogens, Auto and Diesel Exhaust, and Outdoor Air. He Chaired the IARC Monograph on Drinking Water, Food, and Industrial Chemicals (2011). He has published more than 170 articles and received the Alexander Hollaender Award from the EMGS in 2011.

He has mentored 20 graduate students and postdocs through his adjunct professorship at UNC-Chapel Hill. His research interests are molecular mechanisms of mutagenesis, mutation spectra, complex mixtures, and biomarkers of mutation in humans.

Platform Presentations

T1

What's in a Tipping Point? Using Systems Biology to Characterize Adverse Oxidative Responses in Human Lung Cells

Jenna M. Currier[1,2], Wan-Yun Cheng[1,2], Rory Conolly[1], and Brian N. Chorley[1]

[1]US EPA, Research Triangle Park, NC 27711
[2]ORISE Internship/Research Participation Program

Key event-based points of departure distinguishing adaptive and adverse cellular processes can assist the prediction of adverse health outcomes associated with environmental exposures. Here, we used a systems biology approach to characterize cellular responses of the tracheobronchial airway to a model oxidant exposure, zinc (Zn^{2+}). A pharmacokinetic model for normal human bronchial epithelial (BEAS-2B) cells exposed to Zn^{2+} and pyrithione, an ionophore facilitating cellular uptake, was developed to predict the saturation of intracellular Zn^{2+} sequestration necessary for eliciting adverse oxidative effects leading to apoptosis. Computational simulations predicted that 2–10 μM Zn^{2+}/1 μM pyrithione would increase free intracellular Zn^{2+}. BEAS-2B cells exposed to 2–10 μM Zn^{2+} elicited concentration- and time-dependent cytotoxicity. Normal (unexposed), adaptive (2 μM Zn^{2+}), and apoptotic (3 μM Zn^{2+}) exposure conditions were then characterized by assessment of NRF2 and p53 pathway activation. Differences in global gene expression under these conditions were assessed to delineate underlying molecular mechanisms. After 4 h, 154 genes were differentially expressed ($p<0.01$) between the adaptive and apoptotic Zn^{2+} concentrations. Further bioinformatic analyses identified 67 genes associated with p53 and/or NRF2 signaling pathways. This work suggests that the switch between adaptation and apoptosis in our model begins at exposures of approximately 3 μM Zn^{2+} and as early as 4 h after exposure. Future work will identify key event-based points of departure for adverse (versus simply adaptive) events to support expansion of the Zn pharmacokinetic model to assist prediction of adverse outcomes. *This abstract does not necessarily reflect the policy of the US EPA.*

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Platform Presentations

T2

Tobacco Smoke-Associated DNA Methylation and Gene Transcription in Human Blood Cell Lineages

Dan Su, Neal A. Englert, Brian Bennett, Xuting Wang, Devin Porter, Chris Crowl, Michelle R. Campbell, Ma Wan, Gary S. Pittman, and Douglas A. Bell

NIEHS-National Institutes of Health, Research Triangle Park, NC USA

The damage by tobacco smoking to blood cells and their precursors may be subclinical and slowly progressive. DNA methylation profiles in blood have been actively pursued as biomarkers for etiology and risk of smoking-associated disease. However, it is largely unknown how smoking affects genome-wide DNA methylation and gene transcription across immune cell types. Using 450K methylation array, we identified 4784 CpGs that were differentially methylated between 20 smokers and 14 nonsmokers in 4 blood lineages ($p<0.007$). Hierarchical clustering indicated that the overall smoking effect on methylation in monocytes is more similar to granulocytes than to lymphoid B and T cells. Many novel CpGs whose methylation are smoking associated, have linkage to disease (cancer, diabetes, asthma, cardiovascular, arthritis, etc) development, which could be candidate lineage –specific etiological biomarkers for smoking. Gene expression analysis with Affymetrix human transcriptome array unveiled significant smoking-associated coding and noncoding RNA changes in monocyte, granulocyte, B cell, CD4+ T, CD8+ T, and CD56+ NK-cells (fold change >1.7; $p<0.05$), representing novel targets (TWEAK signaling etc) linked to the detrimental effects of smoking. We observed both concordant and cell-specific smoking-associated DNA methylation across immune cell types, and demonstrated, for the first time, cell-type and locus dependency of changes in DNA methylation versus change in gene expression, which has important implications for other exposure studies. The results provide insight into the impact of smoking–associated epigenetic change on gene transcription and immune function for better understanding of tobacco-associated disease risk.

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Platform Presentations

T3

Genome-Wide DNA Methylation Changes Link Cigarette Smoke to Atherosclerosis in Human Circulating Monocytes

Ma Wan[1], Brian D. Bennett[1], Michelle R. Campbell[1], Devin K. Porter[1], Chris L. Crowl[1], Dan Su[1], Neal A. Englert[1], Gary S. Pittman[1], Lindsay M. Reynolds[2], Yongmei Liu[2], Douglas A. Bell[1]

[1]Genome Integrity and Structural Biology Laboratory, NIEHS, RTP, NC, 27709
[2]Wake Forest University School of Medicine, Winston-Salem, NC, 27157

Cigarette smoke associates with numerous human diseases and influences DNA methylation. However, the biological links between DNA methylation changes and disease are largely missing. Here, we identified genome-wide smoking-associated differential methylated regions (SM-DMRs) using Reduced Representation Bisulfite Sequencing (RRBS) of primary monocyte DNA from 58 age, gender and race matched smokers and nonsmokers selected from the prospective Multi-Ethnic Study of Atherosclerosis (MESA). We found that SM-DMRs preferentially occurred at regulatory regions, particularly at enhancers. These SM-DMRs were replicated by RRBS in an independent group of 20 smokers and 20 nonsmokers recruited through the NIEHS. We found 26 significant SM-DMRs shared by both populations with ≥ 5% methylation difference between smokers and nonsmokers, including 20 novel SM-DMRs. Surprisingly, more than one fourth of the 26 top SM-DMRs were close to genes suggested to play a role in monocyte-involved atherosclerosis. We validated the most significant SM-DMR located in an intragenic enhancer of Aryl-hydrocarbon Receptor Repressor (*AHRR*) gene by targeted bisulfite amplicon sequencing and uncovered a nearby CpG site that was more sensitive to smoking exposure than the most significant one (cg05575921) previously identified. Functionally, *AHRR* SM-DMR significantly associated with increased enhancer RNA and *AHRR* mRNA expression in smokers. Importantly, analysis of the cg05575921 CpG site within *AHRR* SM-DMR using 450K array data in 1264 MESA subjects significantly ($p<0.01$) mediated the associations between smoking exposure and subclinical atherosclerosis indicated by ultrasound-measured carotid plaques. Our findings demonstrate that RRBS provides a powerful way to identify SM-DMRs and to link them to smoking-related disease.

NOTES:

Platform Presentations

T4

Two Simulated-Smog Atmospheres with Different Chemical Compositions Produce Contrasting Mutagenicity in *Salmonella*

Jose Zavala[1], Jonathan Krug[2], Sarah H. Warren[3], Q. Todd Krantz[3], Charly King[3], Stephen H. Gavett[3], Michael Lewandowski[2], William A. Lonneman[2], Tad E. Kleindienst[2], Matthew Meier[4], Mark Higuchi[3], M. Ian Gilmour[3], David M. DeMarini[3]

[1]ORISE Fellow, NHEERL, US EPA, RTP, NC 27711, [2]NERL, US EPA, RTP, NC 27711, [3]NHEERL, US EPA, RTP, NC 27711, [4]Health Canada, Ottawa, Ontario, Canada

Ozone (O_3), particulate matter (PM), and nitrogen dioxide (NO_2) are criteria pollutants used to evaluate air quality. Using a smog chamber with UV bulbs to simulate solar radiation, we generated 2 simulated-smog atmospheres (SSA-1 & SSA-2) with different concentrations of these criteria pollutants to explore their mutagenicity. For SSA-1, we added 6 parts per million carbon (ppmC) α-pinene, 24 ppmC gasoline, and 0.5 ppm nitric oxide (NO) continuously into the chamber with nebulized ammonium sulfate (2 $\mu g/m^3$) to provide a nucleation base for secondary reaction products. The photo-oxidized atmosphere produced 97 ppb O_3, 244 ppb NO_2, and 1.07 mg/m^3 $PM_{2.5}$. For SSA-2, adding 6 ppmC isoprene, 9 ppmC gasoline, and 0.9 ppm NO, produced an atmosphere with 440 ppb O_3, 586 ppb NO_2, and 55 $\mu g/m^3$ $PM_{2.5}$. Plates of *Salmonella* TA100 ± S9 were exposed for 0-14 h. Both SSA-1 and -2 were mutagenic only with the lights on and did not require S9. Thus, all the mutagenicity was direct-acting and did not require metabolism. SSA-2 was ~3 times more mutagenic than SSA-1; however, SSA-2 was cytotoxic past 3 h of exposure. O_3 at 440 ppb was not cytotoxic or mutagenic; therefore, the observed results with SSA-2 were due only to other secondary reaction products. Using Next-Gen sequencing, the SSA-1-induced mutants were 50% G to T and 50% G to A. This study shows that secondary reaction products, which are typically not measured, play a key role in air-pollution mutagenicity. [Abstract does not reflect the views/policies of the U.S. EPA.]

NOTES:

Poster Presentations

P1

Uncovering the Most Significant Cigarette Smoking Responsive CpGs Within the AHRR Enhancer Using Bisulfite Amplicon Sequencing in Primary Monocyte DNA and Saliva DNA

Chris L. Crowl , Ma Wan, Devin K. Porter, Michelle R. Campbell, Ryan C. Gimple, Dan Su, Kelly N. Adamski, Gary S. Pittman, Douglas A. Bell

National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC, USA

While it has been shown that smoking cigarettes can lead to significant changes in DNA methylation, the link between these methylation changes and disease is largely unknown. Using 450K methylation arrays, we have recently shown that hypomethylation at cg05575921 in primary monocyte DNA from 1264 participants in the Multi-Ethnic Study of Atherosclerosis (MESA) is associated with subclinical atherosclerosis ($p=3.1\times10^{-10}$) and cigarette smoking ($p=6.1\times10^{-134}$, current vs never smoker), suggesting that hypomethylation at this locus represents a potential biomarker for smoking status and disease risk. Because cg05775921 lies in an intragenic enhancer region of AHRR along with many other neighboring CpGs not captured by 450K, we used Bisulfite Amplicon Sequencing (BSAS) to investigate methylation changes at cg05575921 along with 13 neighboring CpGs in primary monocytes from 43 smokers and 34 non-smokers. While we were able to recapitulate the results from the MESA study regarding cg05575921 ($p=3.87x10^{-15}$, $R^2=0.95$, 450K vs BSAS), we also uncovered novel, significant methylation changes at neighboring CpGs within this enhancer region that are more sensitive to smoking than cg05575921. Furthermore, we used the same technique and region to investigate DNA methylation changes in saliva DNA from 37 smokers and 39 non-smokers. For participants where both monocyte and saliva DNA was assessed, the results for saliva DNA correlate very strongly with the results from monocyte DNA at each of the significantly hypomethylated CpGs. These results suggest that saliva DNA may be a convenient sample for assessing smoking exposure status due to its non-invasive collection.

NOTES:

Poster Presentations

P2

Smoking-Induced Changes in Hematopoiesis and DNA Methylation

Neal A. Englert, Michelle Campbell, Chris Crowl, Gary Pittman, Devin Porter, and Douglas A. Bell

Environmental Genomics Group, Genome Integrity and Structural Biology Laboratory, National Institute of Environmental Health Sciences, Durham, NC 27709

The cellular response to cigarette smoke (CS) involves activation of the aryl hydrocarbon receptor pathway and immune response. CS exposure is associated with reduced DNA methylation in the aryl hydrocarbon receptor repressor (*AHRR*) gene at cg05575921 and at nearby CpGs and is correlated with increased *AHRR* expression. To better understand CS-induced DNA methylation changes during hematopoiesis, we developed an *ex vivo* model of monocyte differentiation to terminal effector cells. We hypothesized that CS-induced methylation changes in *AHRR* in precursor cells would be maintained through terminal differentiation processes. Circulating monocytes from 4 NS and 6 SM volunteers were differentiated for 5 days *in vitro*; resulting monocyte precursor, macrophage, and dendritic cell populations were separated by FACS, and DNA methylation and transcript expression analyses were performed. Distributions of cell populations indicated that SM tended to have a lower percentage of dendritic cells compared with NS. Methylation levels of *AHRR* in macrophages were reduced over 40% in SM compared with NS, indicating methylation differences remain consistent through differentiation processes. Additionally, *AHRR* expression levels in both monocytes and macrophages were increased in SM compared to NS. These data indicate that macrophages maintain epigenetic characteristics of the parent monocyte cells and may thus exhibit CS-induced altered cellular functions. Our data support the hypothesis that CS-induced methylation differences at *AHRR* cg05575921 are maintained through differentiation. Continued studies include reduced representation bisulfite sequencing of FACS cell populations for differentially methylated regions of the genome (NS vs. SM) and expression analysis of transcript levels using microarray-based technologies.

NOTES:

Poster Presentations

P3

Effects of Diethylstilbestrol on Male Reproductive Phenotypes in Genetically Diverse Mice.

Carlee D. Hemphill, Haley R. McCracken, Nicole E. Carns, David L. Aylor

North Carolina State University, Department of Biological Sciences, Genomic Sciences Graduate Program, Center for Human Health and the Environment, Raleigh, NC 27695

Diethylstilbesterol (DES) is a synthetic estrogen that was prescribed to pregnant women from 1940 to early 1970s to reduce the risk of pregnancy complications. This chemical, along with other endocrine disrupting chemicals, have been reported to induce negative effects on developing reproductive systems. Specifically males that were exposed in utero are found to have higher incidences of cryptorchidism, underdeveloped testes, testicular cancer, low sperm counts, and decreased sperm quality. Genetics plays a critical role in controlling how individuals to their environments, and, we observed differential susceptibility to DES exposure across eight inbred mouse strains. We measured reproductive phenotypes of 12-week-old male mice exposed to DES on postnatal days 1-5, which is a widely studied exposure paradigm for DES. We analyzed reproductive organ weights and sperm counts to characterize the effect of DES exposure on reproductive phenotypes. We found a decrease in testis weight, seminal vesicle weight, epididymis weight, and sperm count in DES-exposed litters. Additionally, effects of DES exposure on development manifested in a strain-dependent fashion. Also, we tested the effect of DES on crossover rate during the pachytene stage of meiosis I. Estrogenic compounds have been shown to decrease crossing over in male mice. In order to count crossovers, we isolated individual spermatocytes on slides and stained for the double-strand break marker MLH1, a crossover associated protein. Our research establishes widespread phenotypic variation in DES-exposed inbred mouse strains, and these approaches will enable us to identify specific gene-by-environment interactions associated with DES susceptibility.

NOTES:

Poster Presentations

P4

Transgenerational Effects of Early Life Starvation on Growth, Reproduction, and Stress Resistance in *Caenorhabditis elegans*

Meghan A. Jobson[1,2], James M. Jordan[1], Moses A. Sandrof, Jon D. Hibshman, Ashley L. Lennox and L. Ryan Baugh

Department of Biology, Duke University, Durham, North Carolina, 27705

[1]These authors contributed equally to this work.

[2]Current Address: University of North Carolina School of Medicine, Chapel Hill, North Carolina, 27516

Starvation during early development can have lasting effects that influence human disease risk. We determined the long-term phenotypic consequences of starvation during early larval development in *C. elegans* to develop it as a model for mechanistic studies. Worms recovering from extended starvation grow slowly, taking longer to become reproductive, and are smaller as adults. Fecundity is also reduced, with the smallest individuals most severely affected. Feeding behavior is impaired, possibly contributing to deficits in growth and reproduction. Previously starved larvae are more sensitive to subsequent starvation, suggesting decreased fitness even in poor conditions. The progeny of starved animals are also adversely affected: there is a high incidence of morphologically abnormal embryos with reduced hatching efficiency, progeny are smaller upon hatching and after 48 hr of larval development, brood size is reduced and the incidence of males is increased. However, the progeny and grandprogeny of starved larvae are more resistant to starvation. In addition, the progeny, grandprogeny and great-grandprogeny are more resistant to heat, suggesting epigenetic inheritance of acquired stress resistance. In summary, our results indicate that starvation affects a variety of life-history traits in the exposed animals and their descendants, some presumably reflecting fitness costs but others potentially adaptive.

NOTES:

Poster Presentations

P5

Zinc-mediated Alteration of Cell Cycle in Human Bronchial Epithelial Cells *In Vitro*

Arjun M. Keshava[1], Brian N. Chorley[2], and Jenna M. Currier[3]

[1]Wake Early College of Health and Sciences, Raleigh, NC
[2]NHEERL, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC
[3]ORISE Internship/Research Participation Program

Zinc (Zn^{2+}), a ubiquitous ambient air contaminant, presents an oxidant challenge to the human lung and is linked to adverse human health effects. To further elucidate the adaptive and apoptotic cellular responses of human airway cells to Zn^{2+}, we performed pilot studies to examine cell cycle perturbation upon exposure using a normal human bronchial epithelial cell culture (BEAS-2B). BEAS-2B cells were treated with low (0, 1, 2 µM) and apoptotic (3 µM) doses of Zn^{2+} plus 1 µM pyrithione, a Zn^{2+}-specific ionophore facilitating cellular uptake, for up to 24 h. Fixed cells were then stained with propidium iodine (PI) and cell cycle phase was determined by fluorescent image cytometry. Initial results report the percentage of cells in the S phase after 18 h exposure to 1, 2, and 3 µM Zn^{2+} were similar (8%, 7%, and 12%, respectively) compared with 7% in controls. Cells exposed to 3 µM Zn^{2+} increased cell populations in G2/M phase (76% versus 68% in controls). Interestingly, exposure to 1 µM Zn^{2+} resulted in decreased (59%) cells in G2/M. While preliminary, these pilot studies suggest Zn^{2+} alters cell cycle in BEAS-2B cells, particularly in the G2/M phase. The G2/M checkpoint maintains DNA integrity by enabling initiation of DNA repair or apoptosis. Our findings suggest that the adaptive and apoptotic responses to Zn^{2+} exposure may be mediated via perturbation of the cell cycle at the G2/M checkpoint. *This work was a collaborative summer student project. The studies do not necessarily reflect the policies of the US EPA.*

NOTES:

Poster Presentations

P6

Ablation of C/EBPβ in Oncogenic Ras Tumors Results in Tumor Regression

Zachary J Messenger[1], Robert C. Smart[1,2]

[1]Toxicology Program, Department of Biological Sciences and [2]Center for Human Health and the Environment, North Carolina State University, Raleigh, NC 27695

Ras is a small membrane bound GTPase which is mutated to an oncogenic form in up to 30% of all human cancer. The bZIP transcription factor C/EBPβ is activated downstream of oncogenic Ras, and is required for oncogenic Ras-mediated skin tumor development. To determine if C/EBPβ is required for tumor maintenance and survival, and if the ablation of C/EBPβ results in synthetic lethality in tumors we generated mice in which C/EBPβ could be ablated in preexisting oncogenic Ras containing skin tumors induced by the chemical carcinogen DMBA. Deletion of C/EBPβ in adult mouse skin exhibited no noticeable phenotype, however ablation in oncogenic Ras containing skin tumors resulted in rapid tumor regression where tumor volume was reduced by 95% and tumor multiplicity was reduced by 80% by 8 weeks. The regression was associated with increased levels of apoptosis and p53 protein. To determine if tumor regression was dependent on p53 we generated mice in which p53 and C/EBPβ could be co-ablated in tumors. Tumors in which C/EBPβ and p53 we co-ablated failed to regress demonstrating that tumor regression in C/EBPβ depleted tumors is p53 dependent. In summary, our findings demonstrate that ablation of C/EBPβ in oncogenic Ras driven tumors results in the activation of the anti-tumor activity of p53 which results in rapid tumor regression. These results suggest C/EBPβ could be a molecular target for cancer therapy involving oncogenic Ras tumors.

NOTES:

Poster Presentations

P7

Defining the Biological Domain of Applicability of Adverse Outcome Pathways Across Diverse Species: The Estrogen Receptor/Aromatase Case Study

Maureen Pittman[1], Carlie Lalone[2], Dan Villeneuve[2], Gerald Ankley[2], Stephen Edwards[3], Holly Mortensen[4]

[1]EPA Student Contractor, Research Cores Unit, NHEERL/ORD/US EPA Research Triangle Park, NC 27711, USA; [2]Mid-Continent Ecology Division, NHEERL/ORD/US EPA, Duluth, MN 55804, USA; [3]Integrated Systems Toxicology Division, NHEERL/ORD/US EPA, Research Triangle Park, NC 27711, US; [4] Research Cores Unit, NHEERL/ORD/US EPA Research Triangle Park, NC 27711, USA.

Aromatase inhibitors (e.g. fadrozole, prochloraz) and estrogen receptor antagonists (e.g. tamoxifen) reduce the circulating concentration of 17β-estradiol, leading to reproductive dysfunction in affected organisms. While these toxic effects are well-characterized in fish and thought to be conserved across mammals, it is of interest to assess conservation of these effects in other taxonomic groups to inform our understanding of cross-species variation in toxicity pathways. Here we explore how the AOP construct can be interpreted across diverse species using a comparative pathway approach.

A set of computational scripts has been developed to retrieve Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway information for a specified input gene list, such as the gene target corresponding to the Molecular Initiating Event (MIE) or Key Events (KE) described in an Adverse Outcome Pathway (AOP). The expanded KEGG gene- pathway annotations for available species are then used as input for comparative analysis, with the goal of identifying shared and unique genes, computing enrichment of pathways, and visualizing KEGG Pathway maps between species.

For this initial case study we have curated genes and pathways related to Estrogen Receptor (ER) antagonism and related Aromatase inhibition. Pathway similarity between species (zebrafish, human, chicken, rat, and fruit fly) was analyzed using Bioconductor tools in R and the publically-available pathway analysis tool EC2KEGG. Our findings illustrate that pathways relevant to the ER/Aromatase AOPs show broad similarity between zebrafish, human, chicken, and rat. We find that fruit fly (*D. melanogaster*) lacks gene orthologs for the estrogen receptor, a key molecule that catalyzes estradiol synthesis. This finding suggests that the ER antagonism and Aromatase inhibition AOPs are not applicable to fruit fly, and may have implications to relevance in other invertebrate species.

Disclaimer: The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

NOTES:

Poster Presentations

P8

Evaluating DNA repair center formation and resolution following chemical-induced DNA damage via p53BP1-associated foci detection

Sean M. Rowley, O. Joseph Trask, Salil N. Pendse, Bin Sun and Rebecca A. Clewell

The Hamner Institutes for Health Sciences, Research Triangle Park, NC, 27709

In response to DNA double strand breaks, p53 binding protein-1 (p53BP1) and DNA repair proteins assemble to form “foci” at the sites of DNA damage. These foci are capable of being detected visually and quantitatively as a marker of DNA damage response (DDR). This presentation describes our current efforts to quantify the formation and resolution of these repair centers in a fibroblast sarcoma cancer cell line, HT1080, stably overexpressing p53BP1 coupled with mCherry fluorescent protein. p53BP1-associated foci were visualized using high content imaging with a 40x/0.95NA objective lens for 24 hours after treatment with neocarzinostatin (NCS), an ionizing radiation mimicking chemical, or etoposide (ETP), a topoisomerase II inhibitor. The p53BP1-mCherry live cell assay was validated against wild-type HT1080 cells that were fixed and stained at various times after chemical treatment. These studies provide insight into DRC kinetics in the presence of genotoxic agents. Individual foci were tracked over time in live cells to evaluate rates of DSB induction and repair using commercially available tracking software. The promise of the high content imaging approach presented here is the ability to measure DNA damage repair kinetics in real time at the individual cell level in a high throughput manner. Current screening assays for DNA damaging compounds rely on late stage cellular responses (chromosomal damage, mutation). This assay improves our ability to identify DNA damaging compounds and evaluate the dose-response for DNA damage and repair, and can help guide decisions about environmental chemical exposures.

NOTES:

Poster Presentations

P9

Aberrant Uterine SIX1 Expression May Promote Uterine Adenocarcinoma Following Neonatal Xenoestrogen Exposure

Alisa A. Suen[1,2], Wendy N. Jefferson[1], Charles E. Wood[4], Elizabeth Padilla-Banks[1], Victoria L. Bae-Jump[3], and Carmen J. Williams[1,2]

[1]Reproductive and Developmental Biology Laboratory, National Institute of Environmental Health Sciences, Research Triangle Park, NC, 27709; [2]Curriculum in Toxicology and [3]Lineberger Cancer Center, University of North Carolina-Chapel Hill, NC, 27599; [4]National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Durham, NC, 27709.

Exposing mice on neonatal days 1-5 to the synthetic estrogen, diethylstilbestrol (DES; 1 mg/kg/day), or the phytoestrogen, genistein (50 mg/kg/day), results in high incidences of uterine adenocarcinoma by 18 months of age. Prepubertal ovariectomy prevents the cancer phenotype suggesting that endogenous estrogen exposure is necessary for cancer promotion. Sine oculis-related homeobox 1 (SIX1) is a cancer-associated transcription factor that is permanently upregulated in the uterus after these exposures. Here we investigate the role of aberrant uterine SIX1 expression in the development of xenoestrogen-induced mouse uterine adenocarcinoma and SIX1 as a biomarker in human endometrial cancer. We found that *Six1* mRNA expression was dramatically increased at 6, 12, and 18 months of age in uteri from mice neonatally treated with DES or genistein and positively correlated with uterine adenocarcinoma development. Furthermore, immunohistochemical staining showed that SIX1 protein localized to all uterine hyperplastic and neoplastic lesions. Prepubertal ovariectomy of neonatally DES treated mice resulted in significantly lower *Six1* mRNA expression than in neonatally DES treated intact or adult ovariectomized mice. These findings suggest that uterine SIX1 expression is initiated by neonatal xenoestrogen exposure and perpetuated by endogenous estrogen. In clinical samples of human endometrial cancers, we found a positive correlation between SIX1 expression and endometrial cancer incidence. These findings indicate that uterine SIX1 expression is a disease biomarker and suggest that SIX1 may play a role in uterine carcinogenesis in mice and women. We are currently using mouse genetic models to investigate if SIX1 is necessary or sufficient for cancer development.

NOTES:

Poster Presentations

P10

DNA Methylation Modifies Urine Biomarker Levels in 1,6-Hexamethylene Diisocyanate Exposed Workers: A Pilot Study

Kathie Y. Sun[1], Leena A. Nylander-French[1], Michael C. Wu[2], John E. French[3], Jayne Boyer[1], Lisa Smeester[1], Alison P. Sanders[1,4], Rebecca C. Fry[1]

[1]Department of Environmental Sciences and Engineering, Gillings School of Global Public Health, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; [2]Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA;[3]TOXGEN/Toxicogenetics, Chapel Hill, North Carolina, USA; [4]Department of Preventive Medicine, Icahn School of Medicine at Mount Sinai, New York, New York, USA

DNA methylation may mediate inter-individual responses to chemical exposure and, thus, modify biomarker levels of exposure and effects. We analyzed inter-individual differences in inhalation and skin exposure to 1,6-hexamethylene diisocyanate (HDI) and urine biomarker 1,6-hexamethylene diamine (HDA) levels in 20 automotive spray-painters. Genome-wide 5-methyl cytosine (CpG) DNA methylation was assessed in each individual's peripheral blood mononuclear cells (PBMC) DNA using the Illumina 450K CpG array. Mediation analysis using linear regression models adjusted for age, ethnicity, and smoking was conducted to identify and assess the association between HDI exposure, CpG methylation, and urine HDA biomarker levels. We did not identify any CpGs common to HDI exposure and biomarker level suggesting that CpG methylation is a mediator that only partially explains the phenotype. Functional significance of genic- and intergenic-CpG methylation status was tested using protein-protein or protein-DNA interactions and gene-ontology enrichment to infer networks. Combined, the results suggest that methylation has the potential to affect HDI mass transport, permeation, and HDI metabolism. We demonstrate the potential use of PBMC methylation along with quantitative exposure and biomarker data to guide further investigation into the mediators of occupational exposure and biomarkers and its role in risk assessment.

NOTES:

Poster Presentations

P11

Using Gene Expression Data to Screen for Estrogen Receptor α Modulators

Natalia M. VanDuyn[1,2], Brian N. Chorley[2], Raymond R. Tice[3], Richard S. Judson[2], and J. Christopher Corton[2]

[1]ORISE, Research Triangle Park, NC
[2]US EPA, Research Triangle Park, NC
[3]NIEHS, Research Triangle Park, NC

Gene expression data has the potential to significantly contribute to chemical characterization currently based on high throughput screening (HTS) data, and we demonstrate this with a case study relevant to the Endocrine Disruptor Screening Program. We have developed computational methods to identify estrogen receptor α (ERα) modulators in an existing database of whole-genome microarray data. The ERα biomarker consisted of 46 ERα-regulated genes with consistent expression patterns across 7 known ER agonists and 3 known ER antagonists. The biomarker was evaluated as a predictive tool using the fold-change rank-based Running Fisher algorithm by comparison to annotated gene expression data sets from experiments carried out in MCF-7 cells. Using 141 comparisons from chemical- and hormone-treated cells, the biomarker gave a balanced accuracy for prediction of ERα activation or suppression of 94% or 93%, respectively. The biomarker was able to correctly classify 18 out of 21 OECD ER reference chemicals including "very weak" agonists and replicated 105 out of 114 predictions based on the 18 *in vitro* ERα-associated HTS assays currently employed by ToxCast/Tox21. Importantly, the biomarker accurately predicted 48 out of 56 chemicals evaluated *in vivo* with uterotrophic assays. These results demonstrate that the gene expression-based ERα biomarker can accurately identify ERα modulators in MCF-7 cells, could be considered as a potential "Tier 0" screening model prior to ToxCast/Tox21 HTS assays, and possibly as a potential replacement of one or more of the current ToxCast ER assays. *This abstract does not represent EPA policy.*

NOTES:

Poster Presentations

P12

Utilizing SWIFT Text Mining Tool to Address Challenges of a Literature-Based Evaluation of Transgenerational Inheritance of Health Effects

Vickie R.Walker[1], Katherine E. Pelch[1], Stephanie Holmgren[1] , Brian Howard[2], Ruchir R. Shah[2,3], Kristina A.Thayer[1] and Andrew A. Rooney[1]

[1]NIEHS, RTP, NC, and [2]Sciome LLC, RTP, NC and [3]SSS, Durham NC

The National Toxicology Program (NTP) Office of Health Assessment and Translation (OHAT) uses systematic review methodology to assess the evidence that environmental substances cause adverse health effects. The size and complexity of the literature base for a given topic can range from hundreds to tens of thousands of publications leading to substantial time and resource investments. The aim of this report is to illustrate how text-mining approaches can assist in surveying available information for complex questions with large bodies of literature. A current example faced by OHAT, is to examine the extent of the evidence for transgenerational inheritance of health effects, where exposures to an individual may affect multiple generations removed from the original insult. This phenomenon does not have specific indexing (e.g., MeSH) terms and requires the use of text words or phrases that often have multiple contextual meanings. As a result, a PubMed search for transgenerational inheritance yielded over 55,000 publications, but less than 1% are relevant. In this report, we compared a manual curation approach to one utilizing text mining tools to identify and inventory relevant studies. Using machine learning technology and relevancy ranking based on “seed” studies, over half of the manually curated references could be automatically excluded with a recall rate of 93%. These results suggest a potential reduction in screening burden and reduced screening time per reference. Our data suggests that utilizing text-mining tools has the potential to improve work flow and greatly reduce the time required to evaluate a large body of literature.

NOTES:

Poster Presentations

P13

The New Atmospheric Control Unit (ACU) for the CLARIOstar® Provides Versatility in Long Term Cell- Based Assays from Promega and Platypus.

Carl Peters[1], Tracy Worzella[2], Jennifer Fronczak[3]

[1]BMG LABTECH, Cary, NC 27513, [2]Promega, Madison, WI 53711, [3] Platypus Technologies, Madison, WI 53711

Analyses of cell health and cell migration are examples of cell based assays that derive great benefit from the ability to detect changes to these parameters in real time. Real-time detection allows identification of an exact moment in time when a cytotoxic or anti-proliferative change occurs. Furthermore, assessing cell migration in real time allows the determination of migration rate.

The RealTime- Glo™ MT Cell Viability Assay and CellTox™ Green Cytotoxicity Assay were multiplexed together and monitored for 72 hours. The ACU maintained the 37° C and 5% CO_2 environment for cells to remain healthy so that effects of a panel of compounds could be observed. The multifunctional reading capabilities of the CLARIOstar® allow it to detect the luminescent signal of RealTime-Glo™ MT Cell Viability Assay and the fluorescent signal of CellTox™ Green Cytotoxicity Assay as they changed during the time course.

The Oris™ Pro 384 Cell Migration Assay from Platypus Technologies employ their biocompatible gel based approach. The ACU maintained an environment suitable for the health of fluorescently labeled cells which allowed cell migration to be assessed in two ways. First, the biocompatible gel creates a cell free zone in the center of the well. An increase in fluorescent signal is indicative of increased cells migrating into the cell free zone. Second, the CLARIOstar can perform high resolution well scanning. This enables a heat map to be generated which depicts the extent of cell migration.

The CLARIOstar® with ACU keeps cells happy so that these assays can be achieved.

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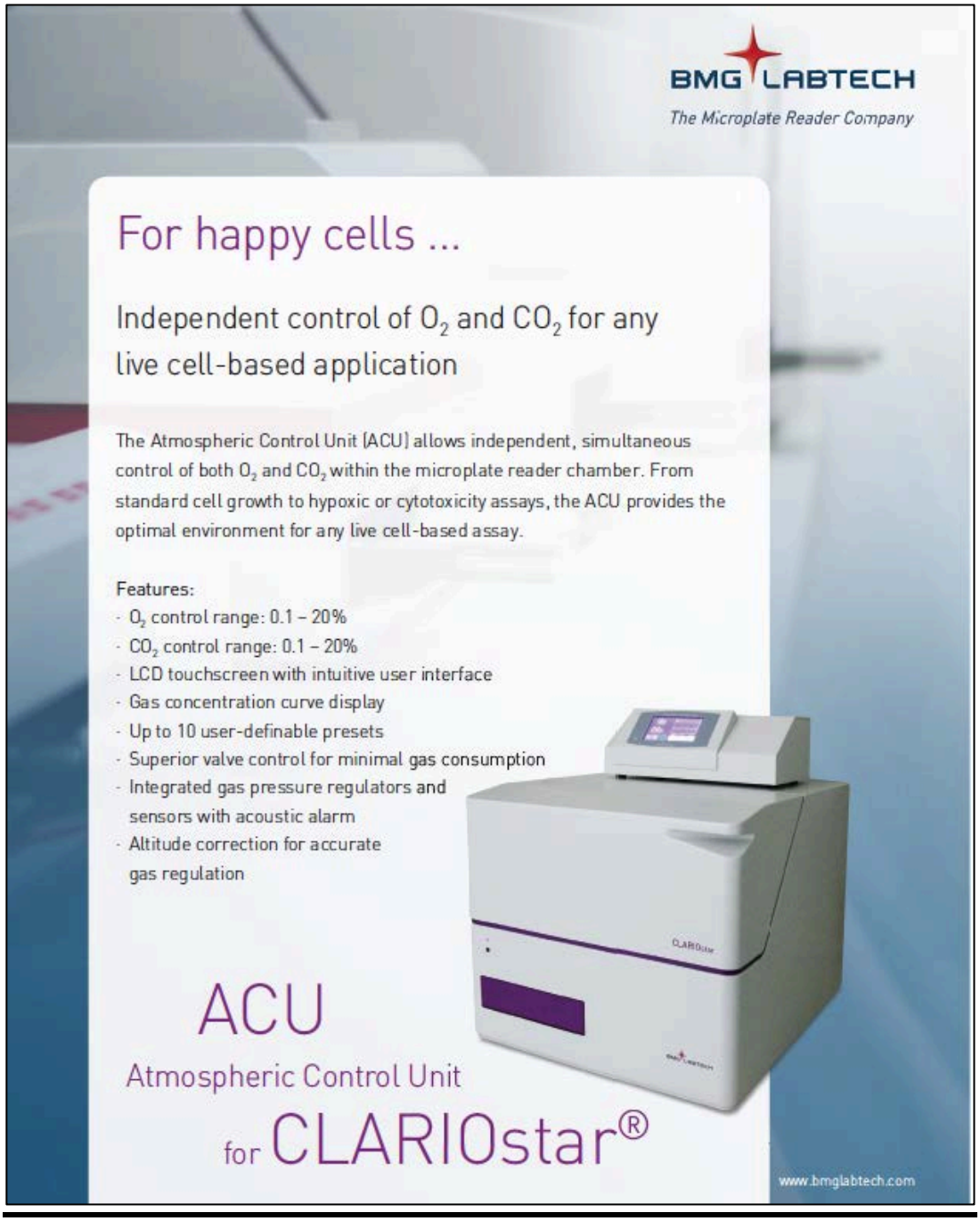

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US EPA
109 TW Alexander Drive
Mail Code: B243-01
RTP, NC 27709
Ph: 919-541-4270
Keshava.channa@epa.gov
keshava.channa4@gmail.com

President-Elect (2014-2015)

Stephanie L. Smith-Roe
Division of the National Toxicology Program at NIEHS
Mailing Address**:**
PO Box 12233, MD K2-17
RTP, NC 27709
Street Address**:**
530 Davis Drive
Morrisville, NC 27560
Room # 2006
Ph: 919-541-4253
stephanie.smith-roe@nih.gov

Secretary (2014-2016)

Holly Mortensen
US EPA
109 T.W. Alexander Drive
RTP, NC 27709
Mail Code: B105-01
Ph: 919.541.2905
mortensen.holly@epa.gov
mortensen.holly@me.com

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Ph: 919-381-7699 (C)
jefrench@toxicogenetics.com

Councilors: 2013-2015

Sarah Warren
US EPA, ORD
109 TW Alexander Drive
Mail Code: B105-03
RTP, NC 27709
Ph: 919-541-0975
warren.sarah@epa.gov
stwarren81@gmail.com

Carol Swartz
ILS, Inc.
P.O. Box 13501
RTP, NC 27709
Ph: 919-281-1110 x814
cswartz@ils-inc.com

Nagu Keshava
US EPA
109 TW Alexander Drive
Mail Code: B243-01
RTP, NC 27709
Ph: 919-541- 3047
keshava.nagu@epa.gov

Councilors: 2014-2016

George Woodall
US EPA
109 TW Alexander Dr.
Mail Code: B243-01
RTP, NC 27709
Ph: 919-541-3896
woodall.george@epa.gov
woodallg@gmail.com

Nancy M. Hanley
US EPA
109 TW Alexander Dr.
Mail Code: B105-03
RTP, NC 27709
Ph: 919-541-7514
hanley.nancy@epa.gov
nanodelle@earthlink.net

Jennifer Nichols
US EPA
109 TW Alexander Dr.
Mail Code: B243-01
RTP, NC 27709
Ph: 919-541-2985
nichols.jennifer@epa.gov
nicoles.jl@gmail.com

Councilors: 2015-2017

Michelle Campbell
NIEHS
111 T.W. Alexander Dr.
Bldg. 101, Rm. C-360, MD: C3-03
RTP, NC 27709
Ph: 919-541-5735
Campbel5@niehs.nih.gov

Erin Pias Hines
US EPA
109 TW Alexander Drive
Mail Code: B243-01
RTP, NC 27711
Ph: 919-541-4204
Hines.Erin@epa.gov

Janice Lee
US EPA
109 TW Alexander Dr.
Mail Code: B-243-01
RTP, NC 27711

Ph: 919-541-9458
Lee.Janice@epa.gov

Member Coordinator:
Carolyn Harris
4107 Kildrummy Ct
Durham, NC 27705
Ph: 919-401-9787
carolynharris@privatedata.com

Corporate Sponsor Coordinator:

Carol Swartz
ILS, Inc.
P.O. Box 13501
Research Triangle Park, NC 27709
Ph: 919-281-1110 x814
cswartz@ils-inc.com

Ex Officio Members:

Thomas Hughes
US EPA
109 TW Alexander Drive
Mail Code: B105-1
RTP, NC 27709
Ph: 919-541-7644
hughes.thomas@epa.gov

Bill Ward (Webmaster)
NHEERL Research Cores Unit, MD105-01
US EPA
RTP, NC 27709
Ph: 919-541-2317
Ward.william@epa.gov
sandbward@mindspring.com

The Genetics and Environmental Mutagenesis Society

- The Society consists predominantly of scientists, students and other interested individuals from private, corporate, government and university organizations, with membership open to all interested parties.

- The goal of GEMS is to promote the study of genetic factors and environmental agents that may pose a genetic risk to humans, and to provide a forum for discussion and interactions among scientists.

- GEMS presents two scientific meetings each year for the membership and guests.

- GEMS provides an opportunity for students and young scientists to become engaged in current scientific topics. Our meetings give young investigators a forum to network with other scientists and to showcase new research. Award winners typically use their grants for attending professional meetings that otherwise they may have been unable to afford.

- Student and post-doctoral trainee GEMS members from government, colleges and businesses in North Carolina are provided an opportunity to hone their presentation skills, meet with senior investigators and peers, and learn more about new trends in biomedical research while competing for juried awards.

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