GEMS

Genetics and Environmental Mutagenesis Society

27th Annual Fall Meeting

"Dissecting Genome Structure, Genetic Traits, and the Basis For Complex Diseases"

Monday, October 5, 2009

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Contents

President's Message

Dear GEMS Members and Guests,

I am extremely pleased to have this opportunity to welcome you to the 27th Annual Fall Meeting of GEMS. The theme of our meeting is "Dissecting Genome Structure, Genetic Traits, and Basis For Complex Diseases." This theme ties together many aspects that have become important in the research of much of our Society's membership. Our President-Elect, Dr. Jef French, has done a truly outstanding job of planning and organizing this meeting, and I wish to express my congratulations and sincere thanks to him for all of his efforts on behalf of GEMS.

GEMS remains a strong and viable scientific society, no small accomplishment in an era of economic uncertainty. This is in no small part due to the efforts of a very talented and dedicated group of GEMS members who comprise the Board of Directors. I have been extremely fortunate to be able to draw upon the time and expertise of this dedicated group, and it has been an honor to work with each of them over the past couple of years. I also want to take this opportunity to encourage each of you to continue to actively support GEMS. If you are not a member, we welcome you to join us. If you are a member, please support GEMS by serving on the Board, by donating your time and talents, by offering your suggestions for improvement, and by encouraging others to join the Society. With all of your help, GEMS will continue to be a strong and relevant organization as we go forward.

I also want to offer my appreciation to all of our Corporate Sponsors, whose ongoing support makes it possible for GEMS to continue to offer high quality scientific programs. Please visit the Sponsors exhibits to discuss with them how they can help you with your research needs. Also, special appreciation goes to NIEHS for supporting this meeting with a generous grant.

Finally, I wish to thank you all for the opportunity to serve as President of GEMS. It has been a rewarding experience for me, and I am honored to have been given the opportunity to help lead the Society. I leave knowing that we have an extraordinary leadership team in place with Jeff French and the Board of Directors to take GEMS confidently into the future.

Sincerely,

Jeffrey Ross
President of GEMS

President-Elect's Message

Dear Members and Guests,

Welcome! First, let me thank the GEMS members and colleagues in the RTP research community who have worked to make this meeting and program possible today. We reached out regionally to many colleges and universities to enlarge our community to provide GEMS support to regional research and education.

A major goal of genetic and genomic research is to understand how the genetic variation between individuals at the sequence and structural levels translate into differences between individuals in disease susceptibility, behavior, toxicity, and many other organism-level traits). To understand the role that genetic and epigenetic differences may play in the evolution of genome architecture and the inheritance of differences in disease susceptibility, etc., we must determine the consequence of evolutionary conserved structural variations have at the population level and the impact at the systems biology level.

In our Spring 2009 meeting, **Michael Resnick (NIEHS)** described how DNA double strand breaks associated with repetitive DNA could reshape the yeast genome and alter structural variation. Using another model organism, **Fernando Pardo-Manuel de Villena (UNC-CH)** illustrated the significant genetic variation at the population level in laboratory-derived and wild-derived inbred strains of mice and how this information was used to establish recombinant inbred lines of mice using 8 different parental lines as a model for complex trait analysis. **David Threadgill (NCSU)** described the role of genetic variation in acetaminophen toxicity in the mouse and human and how candidate genes were identified that were responsible of the observed differences. Finally, **Charles Lee (Harvard Medical School)** illustrated the impact of human structural genomic variation and our incomplete understanding of structural variation in the human genome. Together, these lectures helped set the stage for today's program.

We continue today to examine our present knowledge base on genome architecture of the mouse, a critical research model organism for human disease and the human and the impact of individual genome structural variants in gene expression and disease susceptibility **(Tim Graubert, WUSL and Alex Reymond, UNIL**). This rapidly developing research enterprise also holds the promise of personalized medicine and the challenges and risks the physician faces in interpretation and use, which will be interpreted for us by **Jim Evans, UNC-CH).**

Best regards,

Jef French
GEMS President-Elect

GEMS

Genetics and Environmental Mutagenesis Society

"Dissecting genome structure, genetic traits, and basis for complex diseases"

7:45 Registration and Continental Breakfast & Beverages)

8:30 Welcome: **Dr. Jeff Ross**, GEMS President

Speaker Introduction: **Dr. Jef French**, GEMS President-Elect

8:40 Invited Speaker: **Professor Timothy A. Graubert**, Washington University in St. Louis, *"The impact of copy number variation on local gene expression in mouse hematopoietic stem and progenitor cells"*

9:20 Invited Speaker: **Professor Alexandre Reymond** (University of Lausanne) *"The Influence of Genome Structure on Gene Expression"*

10:00 Poster Session & Visit Sponsors (Morning refreshments in Atrium)

11:30 Lunch (Business meeting during lunch)/Recognition of Past Presidents, Sponsors

1:00-2:30 Selected presentations by Post-Doctoral Students, Technicians, and Students

1:00-1:15 S. Smith-Roe (Abstract T1)

1:15-1:30 B. Chorley (Abstract T2)

1:30-1:45 S. Hunter (Abstract T3)

1:45-2:00 M. McElwee (Abstract T4)

2:00-2:15 R. Jiang (Abstract T5)

2:15-2:30 S. Windham (Abstract T6)

2:30 Invited Speaker: **Professor James P. Evans** (University of North Carolina at Chapel Hill) *"Robust Genomic Analysis; Promises and Problems"*

3:30 Announcement of Awards

4:00 Reception - Visit with Speakers & Sponsors (Refreshments in Atrium)

5:00 Adjourn

GEMS gratefully acknowledges the financial support of the NIEHS Division of Intramural Research DSL Committee for Scientific Conferences in 2009

NIEHS
National Institute of Environmental Health Sciences

Invited Speakers – Brief Biographies

Timothy Graubert, M.D.

Associate Professor
Department of Medicine
Oncology Division
Stem Cell Biology Section
Department of Pathology & Immunology
Washington University in St. Louis
St. Louis, Missouri

Dr. Graubert's research has focused on the molecular pathogenesis of myeloid leukemias. His lab is interested in defining inherited genetic susceptibility factors and somatically acquired mutations important for disease initiation and progression. Specific diseases of interest to his group include: the myelodysplastic syndromes (MDS), acute myeloid leukemia (AML), and MDS/AML arising as a consequence of prior chemotherapy (t-MDS/AML). Both mouse models and correlative studies using human samples are utilized.

To understand the genomics of myelodysplastic syndromes, a large panel of primary human MDS and patient-matched normal (germline) samples have been obtained. Samples are screened for acquired DNA lesions by high-throughput gene resequencing, array-based comparative genomic hybridization, and array-based mRNA/miRNA profiling. Novel mutations are further characterized using tissue culture and mouse model systems.

Susceptibility to therapy-related MDS/AML (t-MDS/AML) is being address by determining the risk for iatrogenic secondary leukemias, which are influenced by inherited genetic susceptibility factors. They are testing the hypothesis in human populations by candidate gene and genome-wide association studies. In addition, they complement this research effort by identification of inbred mouse strains that are susceptible or resistant to t-MDS/AML. Intercross strategies for meiotic mapping are utilized to define the underlying genetic basis of t-MDS/AML susceptibility in mice.

In addition to single nucleotide polymorphisms (SNPs), large-scale DNA copy number gains and losses are an important source of germline genetic variation in mammalian genomes. His lab and colleagues have used array-based comparative genomic hybridization to measure copy number variation in mouse and human genomes. They have also developed novel bioinformatic tools to identify copy number variants, define their boundaries, and relate these gains and losses to phenotypic variation in mice and humans.

Alexandre Reymond, Ph.D.

Associate Professor
Faculty of biology and medicine
Center for Integrative Genomics
University of Lausanne
Lausanne, Switzerland

Alexandre Reymond carried out his thesis research in the laboratory of Dr. Viesturs Simanis at the Swiss Institute for Experimental Cancer Research (ISREC) and received his Ph.D. from the University of Lausanne. After completion of his postdoctoral training with Dr Roger Brent in the Department of Molecular Biology, Massachusetts General Hospital and in the Department of Genetics, Harvard Medical School in Boston, he moved to the Telethon Institute of Genetics and Medicine (TIGEM) in Milan to lead a research group. He joined the Department of Genetic Medicine and Development, University of Geneva Medical School, where he is a member of the Center for Integrative Genomics. His research interests and focus includes human genetics, aneuploidy, Williams-Beuren syndrome, gene expression, genotype-phenotype correlations in human disease.

Besides the millions of individual base-pair changes, termed single nucleotide polymorphisms (SNPs) that distinguish any two unrelated copies of our genome, recent reports have described inversions and copy number variations (CNVs), i.e. large stretches of genomic DNA that vary considerably in copy number amongst individuals. The first map of human copy-number variation reported close to 1500 CNVs covering a total of 360 megabases. This corresponds to as much as 12% of our genome in which hundreds of genes reside. With such prevalence we can readily assume that large-scale size variations play a major role in functional variation and might represent a major force of evolutionary changes. Consistently, numbers of copies of CNVs were recently shown to be associated with multiple human phenotypes (e.g. susceptibility to HIV infection), as well as diseases (e.g. Smith-Magenis syndrome). These effects are brought about through changes in expression levels of the genes that map within the rearranged interval as a function of copy number. Could chromosomal rearrangements affect the phenotype through other means? We recently hypothesized that they might also induce altered expression of the genes that lie near the breakpoints, although the latter do not vary in copy number. This assumption turned out to be accurate as we demonstrated that the human chromosome 7 DNA deletion that causes Williams-Beuren syndrome \ influences the relative transcription levels of some of the genes that map in its flanking regions. Does this effect arise uniquely from loss of genetic material? What about pathological duplications? What about non-pathological polymorphic structural changes? Is this phenomenon restricted to some tissues or effective across different cell lineages and/or developmental stages? These are the questions his laboratory is currently addressing, as well as identifying the mechanisms responsible.

James P. Evans, M.D., Ph.D.
Professor, Department of Genetics
Medical Geneticist
Director, Adult and Cancer Genetics
Director, The Bryson Program for Human Genetics
The University of North Carolina – Chapel Hill
Chapel Hill, North Carolina 27599

Professor Evans interests are concentrated in the field of clinical cancer genetics, pharmacogenomics, and public policy as it relates to genetics. He directs the Clinical Cancer Genetics Services at the University of North Carolina. In the Cancer Genetic Clinic they evaluate and counsel patients who are perceived to be at high risk for cancer by virtue of their personal or family medical history. This comprehensive clinic provides evaluation, counseling and risk assessment through pedigree analysis and genetic testing when appropriate. The clinic has grown substantially since its inception. We now see, on average, approximately 20 patients per week in consultation. While breast/ovarian cancer comprises the bulk of our activity, the staff sees numerous patients with elevated risk for a great variety of different malignancies. Genetic testing is performed in-house for BRCA1/2.

Two of his primary research interests are focused on pharmacogenomics (the study of genetically determined individual response to medications) and attitudes towards the use of genetic information. By defining the genetic underpinnings of drug response one should eventually be able to minimize adverse reactions to medications and improve efficacy. Toward this end they investigate the genetic influences that underlie response to the commonly used anticoagulant, warfarin, and tamoxifen, an agent commonly used in the treatment and prevention of breast cancer.

In addition he is interested in policy issues as they relate to genetics. Towards this end, he has been highly active in science education of the US judiciary at the State Supreme Court and Federal level, as well as at the Supreme Court level internationally. He is a member of the advisory committee to the US Secretary of Health and Human Services on Genetics, Health and Society.

T1

Replication Fork Stabilization Proteins Timeless and Timeless-Interacting Protein (Tipin) Maintain Genomic Stability SL Smith-Roe, PD Chastain II, JJ McNulty, YC Zhou, KA Kaiser-Rogers, M Cordeiro-Stone, WK Kaufmann. Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina.

The Timeless (Tim) and Timeless-interacting protein (Tipin) complex mediates the ATR-dependent intra-S checkpoint in HeLa cells and interacts with replisome factors. We have examined the role of Tim-Tipin in checkpoint activation and DNA replication in normal human fibroblasts (NHF). Depletion of Tim, Tipin, or Claspin by siRNA attenuated UVC-induced activation of Chk1, similarly to depletion of ATR, and depletion of Tim and Tipin reversed UVC-induced inhibition of origin firing, similarly to depletion of Chk1. Cells depleted of Tim, Chk1, or ATR were hypersensitive to UVC exposure in a colony formation assay. In the absence of exogenous DNA damage, depletion of Tim, Tipin, Chk1, or Claspin, but not ATR, compromised BrdU incorporation in a manner suggestive of replication stalling and collapse. ATM, p53, and H2AX were phosphorylated at 24 and 48 h after introduction of Tim, Tipin, or Chk1, but not ATR or Claspin, siRNA. ATR-dependent activation of Chk1 occurred at 24 h in Tim- or Tipin-depleted cells. However, P-Chk1 was not the cause of reduced BrdU incorporation in these cells. Depletion of ATR, Tim, Tipin, or Chk1, but not Claspin, increased frequencies of chromosomal aberrations. A striking defect in sister chromatid cohesion (SCC) accompanied Tim depletion. These findings indicate that Tim and Tipin mediate checkpoint responses to exogenous DNA damage, suppress spontaneous chromosome instability that may arise from failure to stabilize replication forks, and that Tim may promote SCC independently of Tipin. PHS grants ES014635 (WKK) and ES015856 (MCS).

NOTES:

T2

Identifying putative *cis*-acting polymorphisms in NRF2-mediated oxidative stress responsive genes. Brian N. Chorley, Xuting Wang, Michelle R. Campbell, Steven R. Kleeberger, and Douglas A. Bell, National Institute of Environmental Health Sciences, RTP, NC

Nuclear factor (erythroid-derived 2)-like 2 (NRF2) has been identified as a key transcriptional activator of antioxidant enzymes and its absence, as demonstrated in Nrf2 knockout mouse models, leads to damaging effects in tissues with high exposure to pro-oxidants such as lung, liver, and neurological tissues. NRF2 binds *cis*-acting antioxidant response elements (AREs) found in the promoter regions of these genes. We hypothesize that single nucleotide polymorphisms (SNPs) within ARE sequences of NRF2 target genes will affect induced transcription. To assess this, we exposed 60 HapMap CEU cell lines generated from unrelated individuals to the NRF2 activator, sulforaphane, and measured global gene expression with Illumina microarrays. We compared SNP genotype with $\log_2$–transformed expression values for each gene. Among 1027 selected SNPs which were significantly associated with sulforaphane-induced expression (linear regression, $p<0.05$), 23 SNPs were located in gene regions that exhibited NRF2 binding, as demonstrated by ChIP-on-chip. Three of these SNPs were directly within enriched probe regions. This study provides promising SNP candidates for follow-up in molecular validation and clinical disease association studies. Functional SNPs that alter the binding potential and gene transactivation by NRF2 may help explain individual genetic susceptibility to environmental oxidative stressors.

NOTES:

T3

Double Strand Break Repair in Human Mitochondrial Extracts

Senyene E. Hunter, Bennett Van Houten and Joel Meyer, Duke University, Durham, North Carolina

The nature of mammalian mitochondrial DNA (mtDNA) double strand break (DSB) repair is currently not understood. DSB repair was assayed in highly purified human mitoplasts using a newly developed quantitative PCR-based assay that measures ligation across a restriction site. Using this assay, we demonstrate DSB repair carried out by mitochondrial matrix proteins. DNA containing 5' or 3' overhangs is repaired more efficiently than blunt-ended DNA (13%, 8% and 3% repaired, respectively). Further investigation of DSB repair revealed DNA processing, resulting in the loss of approximately 50 bases surrounding the restriction site. Sequence analysis of over 100 products resulted in several patterns of repaired DNA, most with deletions spanning 4-7 bp direct repeats. Results indicate that broken DNA is resected to reveal regions of microhomology, thus allowing annealing and ligation; producing DSB repair-mediated DNA deletions. These deletions are similar to those seen in mitochondrial disorders such as PEO and Kearns-Sayre syndrome. We have determined that DNA polymerase gamma mediates the 3' to 5' resection and is required for the efficient repair of DSBs. Additionally, we hypothesize that the 5' to 3' exonuclease responsible for the resection is hSNM1B. Using immunofluorescence, hSNM1B-GFP is shown to be targeted to mitochondria. Furthermore, the predicted mitochondrial targeting sequence is both necessary and sufficient for mitochondrial localization. Studies are underway to determine the role of hSNM1B in mitochondrial DSB repair. The study of mtDNA DSB repair will help clarify the mechanisms of diseases involving mtDNA deletions between direct repeats that may be associated with DSB repair.

NOTES:

T4

Comparison of Toxicogenomic Responses to Inorganic and Organic Mercury in *Caenorhabditis elegans*. Matthew K. McElwee, Jeff W. Chou, Jonathan H. Freedman. NIEHS, Research Triangle Park, North Carolina

Humans are exposed to methylmercury through consumption of fish, and inorganic mercury through occupational exposures and dental amalgams. While the toxicity of mercury is well established, little is known about the extent to which different species of mercury behave similarly or differently at the cellular level. In order to address this issue, we employed a toxicogenomics approach using the nematode *Caenorhabditis elegans*. We first established equi-toxic responses in *C. elegans* to mercuric chloride ($HgCl_2$) and methylmercury chloride (MeHg) using qRT-PCR of stress response genes and *C. elegans*-specific toxicity assays. Based on these results, we exposed mixed-stage *C. elegans* populations to sub-, low-, and high-toxic mercurial concentrations for 24 h. RNA was isolated from 3 biological replicates from each treatment plus untreated controls, and was hybridized to whole genome Agilent microarrays. Using ANOVA with a Bonferroni post-hoc test ($p<0.01$) and a 2-fold change cut off, we identified 8, 76, and 411 differentially expressed genes in the sub-, low- and high toxic $HgCl_2$ exposures and 44, 424 and 2835 differentially expressed genes in the sub-, low- and high- toxic MeHg exposures. Analysis of the microarray data using principal components analysis, hierarchical and EPIG clustering indicated that *C. elegans* had vastly different transcriptional responses when exposed to different mercury species. These data indicate that there are very large differences in the manner in which different species of mercury affect the cell.

NOTES:

T5

Are Single Nucleotide Polymorphisms (SNPs) Associated with Systemic Dose Critical in Exposure Assessment? Rong Jiang[1], Vandy I. Parron, John E. French, Leena A. Nylander-French [1]Department of Environmental Sciences and Engineering, Gillings School of Global Public Health, University of North Carolina-Chapel Hill

Significant individual variation exists in systemic response to a xenobiotic exposure. The source of variation may be dependent upon single nucleotide polymorphisms (SNPs) in the highly conserved coding region of genes or in their regulatory control sequences (locus-control regions, promoter, intron, etc.) that may affect gene expression and function critical to xenobiotic metabolism. Currently, sources of individual genetic variation in response to exposure to xenobiotics are not accounted for in exposure and risk assessment models. Our goal is to investigate the contributions of individual genetic variations as well as other personal and environmental factors to systemic exposure to naphthalene (*e.g.*, urinary metabolites, skin adducts) among 124 jet fuel workers. SNPs were genotyped using Affymetrix GeneChip Mapping 250K *Sty* array, and SNPs significantly associated with systemic exposure levels were identified using candidate gene and genome-wide association study in PLINK. Multivariate linear regression model was constructed in SAS to investigate the significance of SNPs and other workplace and individual factors. Our results showed that the incorporation of SNP variants increases overall R^2, thus explaining more of the variability in systemic exposure levels than the non-genetic factors without an increase in the number of predictors in the exposure model. This research has allowed us to investigate the impact of individual metabolic differences due to gene expression in response to naphthalene exposure and to increase our knowledge on the potential role of individual genetic differences in exposure assessment. Supported by U.S. Air Force (Texas Tech University subcontract 1331/0489-01), NIEHS P42ES05948, and NIEHS DIR ES021134.

NOTES:

T6

The Effect of Retrospective Sampling on Estimates of Prediction Error for Data Mining Methods to Detect Epistasis Stacey J. Winham and Alison Motsinger-Reif, Department of Statistics, North Carolina State University, Raleigh North Carolina

Positive results from genetic association studies are infamous for their failure to replicate in independent studies, demonstrating the need for more accurate estimates of the predictive value of associations. Recently, a number of novel analytical approaches have been developed for genetic epidemiological studies to identify predictive models that account for complex etiologies. Many data-mining inspired methods are designed to generate testable hypotheses about possible gene-gene interactions to be further investigated by geneticists, and rely on classification error from retrospective case-control data to rank potential models. Previous work has focused on power to detect functional loci, but has not considered the bias and variance of the prediction error estimate derived from classification error. This error estimate is frequently reported, particularly for prediction, but little is known about its statistical properties as both a retrospective and prospective estimator. We evaluate the bias and variance of the error estimate using Multifactor Dimensionality Reduction (MDR) as an example. We show that MDR can both underestimate and overestimate error, in part because of retrospective sampling in case-control studies. We argue that a prospective error estimate is necessary if the model is to be used for prediction, and propose the use of an estimate constructed with bootstrap resampling to accurately estimate prospective error. We show through simulation that this bootstrap estimate is preferable for prediction to the retrospective estimate currently produced. While we use MDR as an illustrative example, the proposed estimation is applicable to all data-mining methods that estimate classification and prediction errors.

NOTES:

P1

Ribosomal Proteins inhibit HDM2MDM2-mediated TP53 ubiquitination from Nucleolar Stresses. Victor Davidson[1], Victor Davidson, Koji Itahana, Checo Rorie, and Yanping Zhang, [1]North Carolina A&T, Greensboro, North Carolina

TP53 is a major contributing tumor suppressor protein that serves to regulate cell cycle and apoptosis. However when TP53 is repressed, mutated, or deleted, it can lead to the development of cancer. HDM2 (MDM2) is a proto-oncoprotein that inhibits p53 TP53 by ubiquitin-mediated degradation and repression of p53 transcriptional activity. Recently we and another group reported that HDM2 (MDM2) interacts with ribosomal proteins L5, L11, & L23 which are prominent ribosomal proteins associated with the 60s large ribosomal subunit. Nucleolar stresses can induce translocation of ribosomal proteins L5, L11, & L23 from the nucleolus to the nucleoplasm, and form complexes with HDM2 (MDM2) to inhibit HDM2 (MDM2)-mediated p53 ubiquitination. To better understand such non-ribosomal functions of L5, L11, & L23, we constructed the Flag-tagged L5, L11, & L23 to isolate their binding partners. In addition, we made EGFP-tagged L5, L11, & L23 to monitor their localization in live cells in response to stress. These constructs will provide us a valuable tool to understand the tumor suppressive pathways regulated by the ribosomal proteins.

NOTES:

P2

DNA Breaks at Fragile Sites Generate Oncogenic *RET/PTC* Rearrangements in Human Thyroid Cells Laura W. Dillon, Manoj Gandhi, Sreemanta Pramanik, Yuri E. Nikiforov, Yuh-Hwa Wang, Wake Forest University School of Medicine, Winston-Salem, NC

Human chromosomal fragile sites are regions of the genome that are prone to DNA breakage, and are classified as common or rare, depending on their frequency in the population. Common fragile sites frequently coincide with the location of genes involved in carcinogenic chromosomal translocations, suggesting their role in cancer formation. However, there has been no direct evidence linking breakage at fragile sites to the formation of a cancer-specific translocation. In this study, we examined the direct involvement of fragile sites in the formation of *RET/PTC* rearrangements, which are commonly found in papillary thyroid carcinomas, and are all a result of a translocation event involving the *RET* gene. These rearrangements are commonly associated with radiation exposure; however most of the tumors found in adults are not linked to radiation. Here, we established that genes (namely *RET*, *CCDC6*, and *NCOA4*) participating in the two major types of *RET/PTC* rearrangements, *RET/PTC1* and *RET/PTC3*, are located in common fragile sites FRA10C and FRA10G. We demonstrated that fragile site-inducing chemicals can induce DNA breakage within *RET/PTC* genes in human thyroid cells by using FISH and ligation-mediated PCR, and by using RT-PCR, that exposure to these chemicals ultimately results in the formation of cancer-specific *RET/PTC* rearrangements. These results provide the first direct evidence for the role of chromosomal fragile sites in the generation of cancer-specific rearrangements in human cells. In addition, this study provides an experimental system to study the effect of environmental and dietary fragile site-inducing chemicals in the generation of carcinogenic chromosomal rearrangements. (Supported by the NCI, CA113863).

NOTES:

P3

Inhibition by Chemopreventive Agents of hCYP1A1 and hCYP1B1 Activation of Benzo[a]Pyrene (B[a]P) and B[a]P(-) 7,8-diol (BPD) to Toxic and Mutagenic Metabolites. Patricia D. Durant[1], Sandra Leone-Kabler[2], Latanya M. Scott[3], Alan J. Townsend[2], Wake Forest University School of Medicine, Winston-Salem, North Carolina

Genetic damage, which can result in changes in function, is believed to lead to a wide range of diseases, including cancer. Benzo[a]pyrene (B[a]P), a polycyclic aromatic hydrocarbon (PAH), is a ubiquitous environmental carcinogen found in tobacco smoke, charbroiled foods and incomplete combustion effluents. Once inside the cell, B[a]P is metabolized to reactive electrophiles capable of binding covalently to protein and DNA, leading to changes in function, genetic damage, mutation, and possibly cancer. Fruits and vegetables are a major source of biologically active compounds called phytochemicals, and are shown to lower the risk of cancer; therefore, they can be classified as chemopreventive agents (CPA). CPA (e.g. resveratrol, genistein, phenethyl isothiocyanate, and galangin) employ several potential mechanisms of protection, including inhibiting cytochrome P450 (CYP)-catalyzed activation of parent PAH compounds to their more genotoxic or cytotoxic metabolites. We investigated whether inhibition of hCYP1A1 or hCYP1B1 activity, as measured by cleavage of the fluorescent VIVID BOMCC substrate in intact cells, was predictive of CPA effects on the cytotoxicity and / or mutagenicity of B[a]P or its intermediate metabolite, B[a]P-7,8-diol (BPD). Inhibition of CYP activity was generally more potent in cells expressing hCYP1B1 than in cells expressing hCYP1A1. However, while the activity inhibition assay was predictive of protection by CPA against the parent compound B[a]P, the same CPA were unexpectedly less effective for prevention of CYP activation of the intermediate metabolite BPD as reflected in the cytotoxicity and mutagenicity assays. Thus, e.g., resveratrol significantly reduced the cytotoxicity and mutagenicity of B[a]P, but not BPD. Our subsequent studies aim to determine the mechanism of the differential inhibition of CYP activation of B[a]P vs. BPD by CPA, as reflected in the metabolic distribution profiles of the parent compound and its intermediate metabolite.

NOTES:

P4

Non-random loss of heterozygosity in γ-radiation induced mouse tumors is mouse strain dependent. John E. French, Vandy Parron, Wilson Chong, and Charles Lee, NIEHS, NTP, RTP, NC

Coordination of DNA strand break repair pathways is dependent on the phase of the cell cycle, DNA replication, and expression of the p53 tumor suppressor protein. Exposure to mutagenic carcinogens results in a significant increase in the loss of heterozygosity (LOH) in p53 haploinsufficient mice. The magnitude of LOH and the rate of DSB repair in response to ionizing radiation appears greatest in C57BL/6J (B6) > C3B6F1 > C3H/HeJ > D2B6F1 > DBA/2 (D2) mice. D2 alleles also appear to suppress both LOH and tumor prevalence. We analyzed both allele specific LOH on chromosome 11 bearing the *Trp53* locus and whole genome LOH using SSLP and aCGH, respectively. Using genomic analysis we identified nonrandom LOH associated with loss of *Trp53, Rad51c*, and *Melm3* on chromosome 11. Using primary cultures of HSC from B6, C3, D2, and their F1 outcross progeny with B6 isogenic mice, we have observed significant strain or genetic differences in the abundance of DNA repair gene transcripts up to 3 h post-irradiation. Using flow cytometry, terminal deoxynucleotidyl transferase (TdT) or γH2AX assays to estimate DNA strand breaks. The magnitude and time required to resolve breaks is significantly different between haplotypes of HR and NHEJ genes. Analysis of DSB repair gene haplotypes by similarity matrices indicates that haplotype diversity between isogenic strains is significant. Using genetic variation as a guide, we selected 6 inbred strains to cross with B6.129-*Trp53*^{tm1Brd} N12 males to create p53 haploinsufficient F1 hybrids for haplotype association with ionizing radiation induced tumorigenesis. Experiments in progress to identify mouse allelic variants in DSB repair genes that may aid identification of human orthologs and extrapolation of results to humans.

NOTES:

P5

Construction of copper biosensor in Listeria monocytogenes to monitor status in macrophages Whitney R. Gore, Christopher Rensing, and C. Dinitra White, University of Arizona, Tucson, AZ and North Carolina Agricultural & Technical State University, Greensboro, NC

Listeria monocytogenes is a gram-positive human pathogen capable of survival in macrophages, which are an important component of the immune system. Normally, macrophages undergo oxidative bursts to destroy microorganisms by producing reactive oxygen species (ROS) that damage proteins, lipids, and DNA. Damage by ROS ultimately prevents bacteria cell replication. Copper is an essential metal for all forms of life; however copper can become toxic to cells by generating ROS. Furthermore, high concentrations of copper are extremely toxic to most bacteria. Therefore, the *goal of this study* was to construct a biosensor that could be used to determine whether the concentration of copper was altered in macrophages infected with *L. monocytogenes*. A biosensor capable of responding to copper was constructed by cloning a copper-responsive promoter and regulatory gene in front of a reporter gene in plasmid pGEM-T Easy. Specifically, PCR was used to isolate a putative copper-responsive operon containing *Lmo 1854* (encodes a regulatory gene), *Lmo 1853* (encodes a Cu-ATPase efflux pump), and *Lmo 1852* (encodes a Cu-chaperone) from *L. monocytogenes* 10403S. The target DNA was successfully cloned into the pGEM-T Easy plasmid as verified by agarose gel electrophoresis. In future studies, the biosensor will be used to monitor the copper response in macrophages upon infection with *L. monocytogenes*.

NOTES:

P6

Gene Expression and Mutation Assessment Provide Clues of Genetic and Epigenetic Mechanisms of Oxazepam-induced Liver Tumors in Mice Stephanie A. Lahousse, Mark Hoenerhoff, Jennifer Collins, Thai-Vu T. Ton, Taka Koujitani, Tiwanda Masinde, Hue-Hua L. Hong, Kenneth Tomer, John Bucher, and Robert C. Sills, NIEHS, RTP, NC USA

Oxazepam is a non-genotoxic chemical that belongs to the benzodiazepine family. It is used therapeutically as a sedative-hypnotic and anti-anxiety medication. A National Toxicology Program study found that chronic exposure to oxazepam resulted in a significant increase in the incidence of hepatocellular adenoma and carcinoma in male and female mice. Five hepatocellular adenomas and 5 hepatocellular carcinomas were examined from male mice exposed to 5000 ppm oxazepam and 6 histologically normal livers from control animals to establish a mutational signature for tumors induced by this chemical. PCR analysis was used to detect mutations in beta-catenin. Global gene expression analysis was performed to distinguish patterns of gene expression between normal liver and oxazepam-induced liver tumors. Consistent with previous results, 60% of the oxazepam-induced liver neoplasms had mutations in beta-catenin. Microarray analysis demonstrated the differential expression of 1,513 genes between normal liver and adenoma, 1,976 genes between normal liver and carcinoma, and 269 genes between adenoma and carcinoma. Expression of genes associated with oxidative stress was significantly altered in adenomas and carcinomas compared to normal liver. In addition, gene expression analysis demonstrated a significant decrease in the expression of genes known to be regulated by methylation in human liver tumors or involved in DNA methylation and histone modification. Our gene expression analysis suggested that the formation of hepatocellular adenomas and carcinomas in oxazepam-exposed mice typically involved oxidative stress, alteration of the Wnt signaling pathway, and potential epigenetic alterations.

NOTES:

P7

The Genotoxicity of Titanium Dioxide and Cerium Oxide Nanoparticles In Vitro Raju Y. Prasad, Kathleen Wallace, Alan H. Tennant, Kirk T. Kitchin, Andrew D. Kligerman, Carl F. Blackman
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The use of engineered nanoparticles in both current and future consumer products is steadily increasing. However, the health effects of exposure to these nanoparticles are not thoroughly understood. This study looked at six titanium dioxide and two cerium oxide nanoparticles of various sizes and manufacturers with respect to their genotoxicity. Immuno spin trapping was used to determine oxidative modification of DNA. A subset of particles was tested in human bronchial epithelial cells (BEAS-2B) cultured in KGM defined medium. Trypan blue dye exclusion was used to establish a dose-response curve of cell toxicity. The single cell gel electrophoresis assay was used to assess DNA single-strand breaks in culture after 24 hr treatment with different concentrations of nanoparticles (10, 50, 100, 150 µg/ml). Degussa P25 AEROXIDE® TiO2 (27.5 nm) and Alfa Aesar TiO2 (32 nm) caused significant DNA oxidative damage at concentrations of 100 and 150 µg/ml. Tail DNA was significantly elevated compared to control for Degussa P25 AEROXIDE® TiO2 at 100 µg/ml after 24 hr treatment. The range of average particle size in suspension was significantly larger than the manufacturer's listed size due to substantial agglomeration in the liquid phase. The results suggest that both Degussa P25 AEROXIDE® TiO2 and Alfa Aesar TiO2 (32 nm) caused oxidative damage to DNA. Additionally, the results indicate that Degussa P25 AEROXIDE® TiO2 may be genotoxic at concentrations of 100 µg/ml and higher, potentially due to free radical formation and oxidative stress mechanisms. [Abstract does not necessarily reflect the policies of the U.S. EPA.]

NOTES:

P8

The Regulation of CD44 Expression in GBM Cells

Timothy A. Raines, Waldemar Debinski, and Patrick M. Martin
North Carolina A&T State University, Greensboro, North Carolina and Wake Forest University School of Medicine, Winston-Salem, North Carolina

Glioblastoma multiforme (GBM) is an aggressive brain tumor that demonstrates a high growth rate and the capacity to invade surrounding brain tissue. These characteristics result in a dismal prognosis for GBM patients. Current treatment for GBM tumors involve surgical resection followed by radiation and/or chemotherapy. Disappointingly, many GBM tumors are resistant to radiation and chemotherapy, and the invasiveness of GBM precludes removal of the tumor. GBM cell infiltration has been associated with the transmembrane glycoprotein, CD44, the principle cell surface receptor for hyaluronan. The molecular mechanisms that regulate CD44 in GBM are not fully understood. However, CD44 protein expression has been associated with an AP-1 transcription factor, FOS related antigen 1 (FOSL1), in other cancers. We investigated the regulation of CD44 expression in glioma cells by FOSL1. Human GBM cell lines, U-251 and U-1242, were exposed to either epidermal growth factor (EGF, 100 ng/ml) or hepatocyte growth factor (HGF, 50 ng/ml), and western blot analysis was used to determine the expression of CD44 and FOSL1. CD44 and FOSL1expression levels increased with longer exposures (4 to 24 hr) to EGF and HGF. To determine whether FOSL1 takes part in the regulation of CD44 expression, we utilized siRNA directed against FOSL1. FOSL1-siRNA decreased FOSL1 expression in EGF- and HGF-treated GBM cells. Following HGF stimulation and FOSL1-*1*-siRNA treatments, CD44 expression also decreased. However, EGF stimulation of FOSL1-siRNA treated U-1242 GBM cells showed no change in CD44 expression. These data suggest that FOSL1 regulation of CD44 expression is growth factor-specific in GBM cells.

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P9

Characterization of a putative pariplasmic iron efflux pump in Shewanella strain ANA-3 and S. oneidensis strain MR-1

Lyndsey Reid, Christopher Rensing, and C. Dinitra White
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Shewanella bacteria are of interest in many environmental studies for their metabolic versatility. This bacterial species detoxifies toxic metals in a process termed bioremediation. Shewanella mobilizes iron and other insoluble metals into soluble form during anaerobic respiration. Almost all existing organisms require iron and copper for growth, but at high concentrations these metals become extremely toxic. Therefore cells must regulate their internal metal concentrations. Resistance Nodulation Division (RND) proteins play a role in this heavy metal efflux (HME) system. Examples of RND proteins include the CusCFBA systems of Escherichia coli and other gram-negative bacteria. The purpose of this study was to characterize this efflux system in S. ANA-3 and S. oneidensis MR-1. The minimal inhibitory concentration (MIC) of copper was tested in each strain and S. ANA-3 was determined to have a higher MIC than S. oneidensis MR-1. Genomic DNA was successfully isolated from S. ANA-3 and S. oneidensis, and used in PCR to isolate the cusA gene. cusA was then cloned into a pGEM-T Easy vector which was verified by restriction digest analysis. In future studies, our goals are to further examine cusA by detecting and quantifying cusA transcripts in mRNA of Shewanella ANA-3 and S. oneidensis MR-1 in response to copper and iron exposure. Moreover we plan to fully characterize the remaining genes involved in this important efflux system.

NOTES:

P10

Mitochondrial fusion and autophagy aid in removal of persistent mitochondrial DNA damage Smith, Amanda M., Crocker, Tracey L., Leung, Maxwell C.K., Meyer, Joel N. Duke University, Durham, NC USA

Mitochondrial DNA (mtDNA) integrity is critical for human health; however, it is unclear how bulky mtDNA adducts formed after exposure to environmentally important genotoxins such as ultraviolet radiation and PAHs are handled. mtDNA may be particularly susceptible to these genotoxins due to the absence of nucleotide excision repair, the primary repair mechanism for bulky DNA adducts in nuclear DNA. We investigated the removal of bulky mtDNA adducts via mitochondrial fusion and autophagy in *Caenorhabditis elegans*. Larval fusion, fission and autophagy mutant *C. elegans* were exposed to serial UVC doses over 48 hours. This exposure protocol allows for accumulation of mtDNA damage without lasting damage to nuclear DNA and results in measurable larval growth arrest. Strains carrying mutations in the autophagy gene *atgr-18* and fusion genes *fzo-1* and *eat-3* exhibited exacerbated larval growth arrest with little to no growth recovery after 72 hours. We concluded that these proteins are required for normal recovery from mtDNA damage-induced larval growth arrest. In order to test directly the contribution of autophagy and fusion proteins to removal of UVC-induced DNA damage, we performed RNAi knockdown of autophagy and fusion genes in UVC treated adult *glp-1 C. elegans*. Knockdown of autophagy and fusion genes inhibited removal of UVC-induced DNA damage, as measured by a quantitative PCR-based assay. These data suggest that autophagy and fusion processes are involved in the removal of bulky DNA damage in mitochondria. Mitochondrial dysfunction as a result of bulky mtDNA damage may trigger mitochondrial remodeling. Preliminary results indicate the relative ATP levels are reduced in UVC treated animals. Therefore, we hypothesize that UVC-induced mtDNA damage is removed via fusion-mediated mitochondrial remodeling and subsequent autophagy, possibly triggered by mitochondrial dysfunction. This research was supported by funding from the National Institutes of Environmental Health Sciences, 1 P30 ES-011961-01A1.

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